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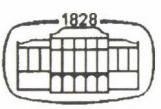

AKADÉMIAI KIADÓ
BUDAPEST

EDITORIAL NOTE

Celebration of the 25th Volume of Acta Alimentaria

This year we shall celebrate the 25th anniversary of the foundation of our journal. On this occasion, we feel prompted to conduct a review on our activities carried out so far, and to establish whether the goals set by our Editorial Board 25 years ago, upon the proposal of the Complex Committee for Food Science of the Hungarian Academy of Sciences then formed, have been successfully achieved.

Our primary aim was to offer experts working in the promising field of food industry, as well as researchers in institutes and university laboratories, the opportunity to publish results attained in their fields, comprising all phases of investigations, from basic research to industrial application.

I feel that, in this respect, the Editorial Board of the journal has done its utmost. At the same time, the opportunity of presenting results to an international forum of experts has also inspired our researchers to meet the requirements of the highest scientific standards.

Our current problem is that, owing to financial difficulties arising during the change of our economic system, the greatest concern of our investigators is, unfortunately, no longer the international acknowledgement of their work. I do hope, however, that after stabilization of the economic situation of research institutes, our Editorial Board will soon be receiving again a great number of papers worthy of publication from industrial sources.

The Editorial Board has gradually introduced also publication of international papers. It is by no chance that our journal has, in the meantime, assumed the subtitle: "An International Journal of Food Science". As a result, an international editorial board has been set up, with distinguished authorities on food science, and international associate editors, who – through their high level of expertise and exacting work of supervision – greatly contribute to preserving, and hopefully, also raising the standards of our journal.

In expressing, on the occasion of this anniversary, grateful thanks to all members of our Editorial Board and to the wide range of contributors, I am asking you to kindly support us in our efforts to maintain and increase the international acknowledgement of our journal also in the future.

J. Holló
Editor

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LACTIC ACID FERMENTATION OF MUSHROOM (*AGARICUS BISPORUS*) FOR PRESERVATION AND PREPARATION OF SAUCE

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Introduction of lactic acid fermentation into mushroom (*Agaricus bisporus*) and preparation of sauce from fermented mushrooms was studied as a method of extension of its postharvest life. Mushrooms (blanched or unblanched) with dry salting, similar to the sauerkraut preparation, did not undergo lactic acid fermentation. The amount of acid produced was negligible (0.03–0.04%) and the pH did not reduce below 4.39 and it was higher than the microbiologically critical value (pH 4.1). Mushrooms of all these treatments got spoiled within 24 h with microbial count ranging from 4.0×10^4 to 1.5×10^8. Even the addition of lactose didn't stimulate lactic acid fermentation in dry salting procedure. Mushroom-in--brine inoculated with lactic acid bacteria produced more acid than the uninoculated mushroom. Amongst the bacteria tried, *Lactobacillus plantarum* produced the highest amount of acid (0.59%) after 5 days of fermentation. The bacterium sharply decreased the pH to less than 4.0 in one day. Two % salt supported the higher acid production than 3%. Fermentation softened slightly the spongy texture of mushrooms without altering the colour and flavour of fermented mushrooms. The fermented mushroom along with brine could successfully be converted into sauce of desirable physico-chemical characteristics, sensory qualities and shelf life. The method of preparation and quality characteristics of mushroom sauce are also described.

Keywords: lactic acid fermentation, mushroom, sauce, preservation

The mushroom is a delicious and nutritious crop but has a short postharvest life and deteriorates in quick succession. It is specially true in the countries with hot and humid climates. Extension of postharvest life of such perishables, is therefore extremely important. Preservation by fermentation is one of the methods available for this purpose. Preservation of food by lactic acid fermentation is an ancient method. It is true especially for vegetables where either natural fermentation or fermentation by pure culture of lactic acid bacteria is practised even in the developed countries (PEDERSON, 1971; ANDERSSON et al., 1990; FLEMING & MCFEETERS, 1981; DAESCHEL & FLEMING, 1984). Lactic acid fermentation removes fermentable

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sugars, prevents growth of pathogenic microorganisms, stabilizes the product and enhances flavour (FLEMING et al., 1983), besides being a cheap and simple technique. Lactic acid bacteria are also known to produce 'bacteriocins' which inhibit the growth of pathogenic microorganisms (HILLIER & DAVIDSON, 1991). The lactic acid fermented foods are safe and are storable without spoilage. Many fermented food products are prepared and consumed all over the world (FRAZIER, 1971) and the techniques for lactic acid fermentation of many fruits and vegetables is well standardized (PEDERSON, 1971; ANDERSSON et al., 1990). However, information on the fermentation of button mushrooms (*Agaricus bisporus*) is scanty except for solitary report on preservation of oyster mushroom by this technique (KREB & LELLEY, 1991). One of the purposes of this investigation was to introduce lactic acid fermentation in button mushrooms either naturally or by the pure culture of lactic acid bacteria. The other objective was to prepare and evaluate sauce from the fermented mushrooms for which at present, no technique is available. The results obtained on these aspects are presented in this paper.

1. Materials and methods

1.1. Microorganisms

The strains of *Lactobacillus plantarum* (2083), *Lactobacillus delbruckii* (2365) and *Streptococcus lactics* (2407) used in the experiment were procured from National Chemical Laboratory, Pune, India. The strains were subcultured periodically at 30 ± 1 °C on the lactic acid bacteria (LAB) agar and maintained in the same medium at 4 °C. The composition of the medium was: Glucose 0.5%, lactose 0.5%; liver extract 0.6%; sodium acetate 0.5%; salt solution A ($K_2H\ PO_4$ 10%; KH_2PO_4 10%) 0.5 ml and salt solution B ($MgSO_4\ 7H_2O$, NaCl, 0.2%; $MnSO_4\ 4H_2O$, 0.2%) 0.5 ml per 100 ml of the medium.

1.2. Mushrooms

Edible mushrooms (*Agaricus bisporus*) used in the study were procured from National Mushroom Centre, Solan (H.P) India.

1.3. Fermentation

The mushrooms, chopped into slices of the size 1×0.1 cm, were either blanched or unblanched. In one set of experiments, they were mixed either with 2.0 or 3.0% salt with or without addition of lactose (1%). After proper mixing these were weighed down in the similar manner as with preparation of the sauerkraut (FRAZIER, 1971). All the treatments were kept at a temperature of 30 ± 1 °C for 24 h.

For the brined fermentation the blanched and chopped mushrooms were covered with hot brine in a flask, plugged with cotton and kept for incubation. The brine was prepared with 2% salt and 1% lactose. The surface of the brine was covered with a layer of mustard oil during storage and kept for observations. The ratio of brine to the mushrooms was kept at 2:1. During fermentation, monitoring of acid production and changes in pH was carried out.

Pure cultures of lactic acid bacteria were used in other experiments. Mushrooms were blanched in boiling water (5 min) and were covered with hot brine (85 °C) of either 2 or 3% concentration and divided into separate sets. Each set was inoculated separately with specific lactic acid bacteria viz., *L. plantarum*, *L. delbruckii* and *S. lactics* and one set with a mixture of strains (*L. plantarum* and *L. delbruckii* in 1:1 ratio). To each set 1% lactose, solution of $MgSO_4$ $7H_2O$ (0.05%) and $MnSO_4$ $7H_2O$ (0.05%) were added, as these salts were reported to be stimulatory for the growth of these bacteria (AHMED & MITAL, 1986). The cultures of each strain were prepared separately in lactic acid bacteria (LAB) broth and their growth was determined by measuring optical density at 650 nm. The optical density of the inoculum was measured with a colourimeter. The respective inoculum were added at the rate of 2% (v/v) to the brine.

The brine was added hot (85 °C) to the sliced mushrooms in the ratio of 2:1 and when the brines cooled, then they were inoculated with the respective cultures. The fermentation was carried out for 5 days at 30 ± 1 °C.

1.4. Preparation of sauce

The fermented mushrooms along with brine were converted into a paste by passing through a mixer. Different ingredients such as spices, salt, sugar etc. were added as per the standard recipes used in the sauce preparation. The sauce was prepared from the paste with the routine method employed in fruit sauce preparation (CRUESS, 1951). The same was cooked to the proper consistency. Acetic acid was added to the sauce. The sauce was preserved with sodium benzoate (750 mg kg^{-1}), bottled and corked followed by pasteurization of bottles in boiling water for 20 min.

1.5. Analyses

During the experiments, titratable acidity (%LA) and pH of fermenting brines were measured for 5 days. The sauce was analysed for TSS (°B), titratable acidity, pH, salt as sodium chloride, standard plate count, relative flow rate and sensory characteristics as per the standard methods (RANGANNA, 1986). TSS was measured with a hand refractometer while pH was monitored with digital El-top pH meter. The colour of mushroom sauce was measured with Lovibond tintometer and were expressed as tintometer units of red, yellow and blue as per the method described

(RANGANNA, 1986). The relative viscosity of the prepared sauce was measured with Ford's viscometer and expressed as flow in min. The product was incubated at 37 °C for a week. A panel of five trained judges evaluated the sample of sauce for the sensory quality characteristics on the hedonic scale. Single sample presentation technique was followed in sensory analysis. Efforts were made to obtain unbiased judgement. All the experiments were done in triplicate. The results of analysis of sauce are reported as average ±SD.

2. Results and discussion

2.1. Dry salting natural fermentation

The results (Table 1) show that neither blanched nor unblanched mushrooms with dry salting undergo lactic acid fermentation rather got putrefied within 24 h. Addition of lactose to the mushrooms along with salt did not have any major effect either on the acidity or the pH except for a few minor differences between the treatments. Lactose addition reduced slightly the pH values in the treatments with lactose than those without it, irrespective of being blanched or not. However, in all the treatments, the pH value did not fall below 4.39. Since the pH of 4.1 is critical for the microbiological stability of the product and on achieving this value microbial spoilage can be prevented. The standard plate count did not indicate any specific pattern with respect to either of salt content, addition of lactose or blanching. But the visual colour of mushroom could differentiate the blanched mushroom from the unblanched as the former had whitish brown colour compared to the dark brown of the unblanched mushrooms. The change in colour is probably the effect of blanching of mushrooms wherein heating followed by immediate cooling is known to inhibit the enzymes responsible for browning in vegetable such as mushrooms, having very high enzymatic activity (HAMMOND, 1978). Similarly blanching of oyster mushroom prior to fermentation successfully prevented softening and discolouration of fruiting bodies (KREB & LELLEY, 1991). All the treatments gave the putrid and highly offensive smell characteristics of the protein decomposition within 24 h, indicating the spoilage of the mushrooms. Microbiological examination did not confirm the occurrence of lactic acid bacteria in the brine. The behaviour of all the treatments with respect to this parameter was identical and could easily be correlated with the pH values discussed earlier. Thus, mushrooms by dry salting did not undergo lactic acid fermentation. Recently, in oyster mushrooms inoculation with brine from sauerkraut fermentation introduced the lactic acid fermentation (KREB & LELLEY, 1991). It indirectly confirms our observation that introduction of lactic acid fermentation by dry salting is not successful in mushrooms.

Table 1

Details of dry salting treatment and some characteristics of mushrooms after fermentation

	Treatment		Titratable acidity (%)		pH		Standard plate count (cfu g^{-1})	
	Salt (%)	Lactose (%)	UB	B	UB	B	UB	B
A)	2.0	0.0	0.04	0.03	5.62	5.32	1.0×10^{6}	1.7×10^{6}
B)	2.0	1.0	0.04	0.03	4.38	4.78	1.5×10^{8}	3.6×10^{5}
C)	3.0	0.0	0.03	0.03	5.80	5.16	1.5×10^{8}	3×10^{7}
D)	3.0	1.0	0.03	0.03	4.88	4.39	7.9×10^{5}	4×10^{4}

UB: Unblanched, B: Blanched. All treatments got spoiled within 24 h.

Fermentation of many vegetables by the natural microflora using dry salting procedure is carried out as a routine method (PEDERSON, 1971). It is a conventional process of sauerkraut preparation. Non-introduction of effective fermentation in salted mushroom could be attributed to non-availability of the fermentable sugars in mushroom in sufficient quantity (STANTON, 1984) to produce enough acid for imparting microbiological stability to the fermented product. Moreover, the protein content of mushroom is high which may have provided buffering action to whatever small amount of acid might have been produced, as observed also earlier (KREB & LELLEY, 1991). No drastic change in pH value of mushrooms further suggests the possibility of the growth of food poisoning and or proteolytic microorganisms in the salted mushrooms.

2.2. Brined fermentation with lactose

Blanched mushrooms in 2% brine and 1% lactose showed acid production and reduction in pH value in the brine progressively (data not shown). Addition of brine to the mushrooms could have provided anaerobic environment conducive to the growth of the lactic acid bacteria in contrast to the dry salting procedure as described above. According to KREB and LELLEY (1991) there is reduction in pH value and growth of lactic acid bacteria though slowly in oyster mushroom without inoculation. To this extent these results confirm our observations. The fermented mushrooms stored with a layer of mustard oil on the surface did not show any visible sign of spoilage. Similarly, no post fermentation change in oyster mushroom fermented by the lactic acid fermentation have been reported (KREB & LELLEY, 1991). Changes such as increase in pH are introduced normally by yeasts or bacteria (LIEPE & JUNKER, 1979). The oil layer on top of the fermented mushroom might have prevented their growth and consequently the changes in the mushrooms. The prevention of spoilage of mushrooms could be associated with the activity of lactic

acid bacteria such as reduction of pH, removal of fermentable sugars (primary preserving actions), production of antibacterial substances like hydrogen peroxide, diacetyl, bacteriocins and reuterin (DAESCHEL, 1989). Various species of bacteria like *Pseudomonas* and *Bacillus* are reportedly inhibited by the activity of lactic acid bacteria (PULUSANI et al., 1979; BELIARD et al., 1989). Besides this, due to the lowering of pH, possibility of growth of food poisoning microorganisms and their occurrence is excluded.

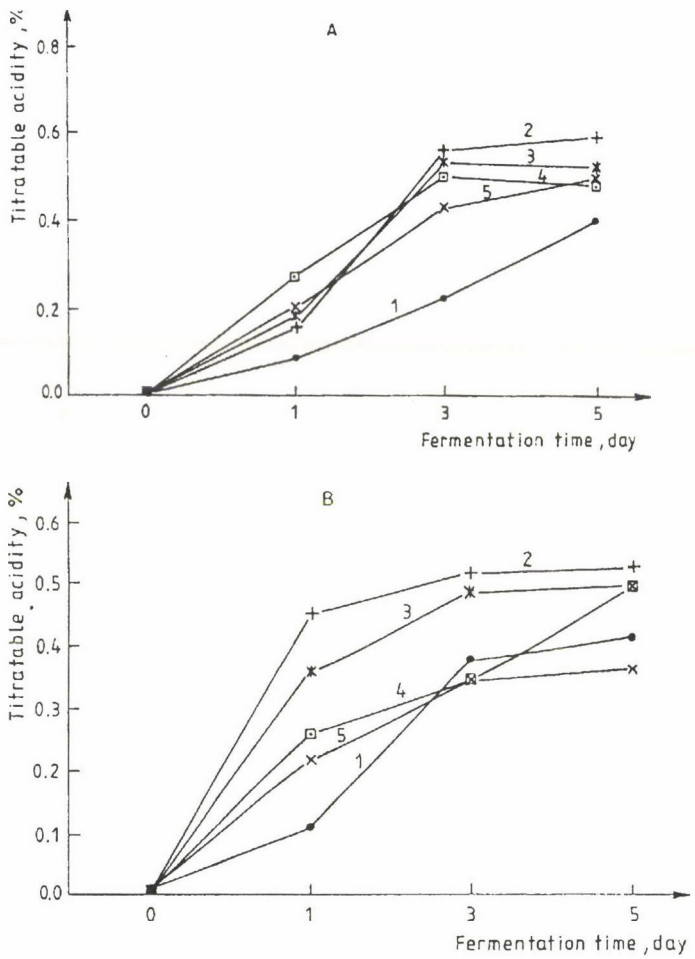

Fig. 1. Changes in acidities of mushroom in brine inoculated with different lactic acid bacteria. A: 2% Brine; B: 3% Brine. 1: Control; 2: *L. plantarum*; 3: *S. lactis*; 4: *L. delbruckii*; 5: *L. delbruckii* + *L. plantarum*

2.3. Lactic acid bacterial inoculated fermentation

Figure 1 illustrates the increase in acidity in all the treatments including controls. Much more acid was produced in the inoculated brines than in the respective controls. The increase in acidity was more with strains of *Lactobacillus plantarum* (0.52%) and *Streptococcus lactic* (0.49) at 2% concentration of salt in 24 h only, as compared to other strains. Similar to these results, addition of brine from sauerkraut fermentation has successfully introduced the lactic acid fermentation for producing a palatable and storable product, where lactic acid was produced along with the growth of *Lactobacillus plantarum*. *Lactobacillus plantarum* produced the highest amount of acid in green olives and cucumbers fermentation also (ETCHELLS et al., 1964; 1966). Natural fermentation of chickpeas and cowpeas produced higher amount of acid than obtained in our study probably due to the addition of more sugar than we tried (ZAMORA & FIELDS, 1979). The production of high acidity in oyster mushroom inoculated with sauerkraut brine has been attributed to the dominance of *Lactobacillus plantarum*. Less production of acid at high salt content might be due to the detrimental effect of high salt concentration on the growth of lactic acid bacteria as reported earlier (PEDERSON, 1971). Out of the two salt percentages, 2% had more acid content than 3%.

Figure 2 shows that there were drastic changes in pH of the brine. It decreased in all the treatments. The decrease in pH was more pronounced in the brines inoculated with pure culture of *Lactobacillus plantarum* or with mixture of *L. plantarum* and *L. delbruckii* than the control or other strains tried. In both the salt concentrations *Lactobacillus plantarum* inoculation, or its combination brought down the pH value below 4.00, within 24 h of fermentation. However, in other strains or the control, the pH value remained above 4.00. After 24 h the differences between the strains were reduced and flattening of the curve could be seen indicating the saturation in the acid production or stability in acidity in various treatments. Nevertheless, the lowest pH was obtained in *Lactobacillus plantarum* fermentation. The pH value below 4.1 is considered safe because food poisoning microorganisms have no possibility of growth. The flattering of pH graph, as seen in our results, has been observed earlier too (KREB & LELLEY, 1991), attributing to the buffering capacity of mushrooms. The trend of decrease in the pH of the inoculated mushrooms was similar to that reported for cowpeas and chickpeas as a result of natural fermentation (ZAMORA & FIELDS, 1979) or the oyster fermentation inoculated with brine of sauerkraut fermentation (KREB & LELLEY, 1991). The final pH values of brines after 5 days of fermentation were almost similar.

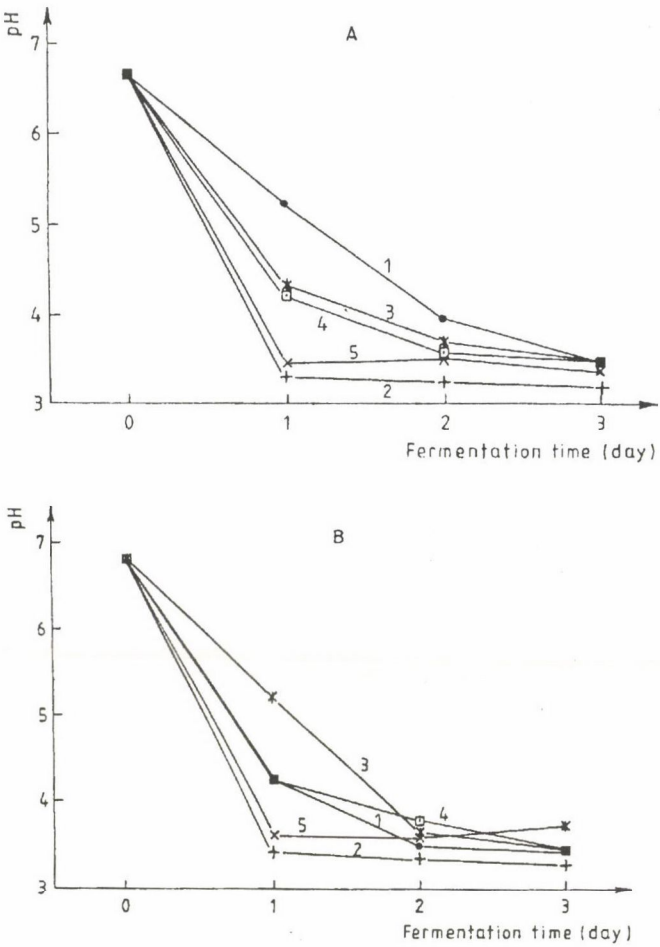

Fig. 2. Changes in pH values of mushroom in brine inoculated with different lactic acid bacteria. A: 2% Brine; B: 3% Brine. 1: Control; 2: *L. plantarum*; 3: *S. lactis*; 4: *L. delbruckii*; 5: *L. delbruckii* + *L. plantarum*

During the fermentation of mushroom, it was observed that the colour of brines changed from colourless to yellowish green. The change could be attributed to the production of hydrogen sulfide as has been reported in meat and cheese (SHARP & FRANKLIN, 1962; LEE & SIMARD, 1984). The colour of mushroom, however, remained unchanged which is desirable. Similarly to these observations fermentation of oyster mushrooms by brine from sauerkraut fermentation did not change the colour of the mushrooms, though they reportedly gave appearance of being washed out (KREB & LELLEY, 1991). The mushrooms and the brine in our studies gave typical mushroom aroma unlike those reported by KREB and LELLEY (1991), where these oyster mushrooms had light smell of sauerkraut along with original flavour of

mushrooms. As expected, the aroma and taste of the fermented mushrooms was acidic, typical of lactic fermentation. The texture of the mushrooms had softened slightly as a result of fermentation. In the lactic acid fermentation of oyster mushrooms the changes in the texture were hardly recognised (KREB & LELLEY, 1991). To prevent the softening, heat processing of fermented mushrooms was recommended (FLEMING et al., 1983).

2.4. Fermented mushroom sauce

The physico-chemical characteristics and sensory qualities of the mushroom sauce prepared from the fermented mushrooms are given in Table 2. According to the limits prescribed TSS and titratable acidity of any sauce should not be less than 15 °B and 1%, respectively. Hence, the product meets the minimum requirements. The addition of sugar to the fermented mushrooms is reflected in the enhanced TSS of the mushroom sauce. Similarly, the difference in the acid content of the fermented mushrooms and the sauce is partly the result of addition of acetic acid as an acidulant and partly to the concentration of acid already present in the paste used in sauce preparation.

The colour of the mushrooms sauce was attractive. There are no standards for this parameters in mushroom sauce. However, the presence of yellow and red colour units in the sauce indicates the attractiveness of the product, the blue units indicate the depth of colour/darkness in the product. Since the sauce is a thick paste, occurrence of the blue colour units is natural. The relative viscosity of the product was found to be desirable. It was thick but free flowing, typical of any sauce. With respect to the salt content, there is no prescribed limit and its quantity observed here is normally found in any sauce. However, salt content is more important with respect to its influence on taste than its absolute value. The microbiological analyses showed the presence of a small number of aerobic microorganisms which could probably be due to the survival of spores, but incubation tests and anaerobic counts being negative indicate desirable microbiological safety (FRAZIER, 1971) and shelf life.

The sensory evaluation score (Table 2) revealed that most of the quality attributes were in the desirable range. The colour of the product was described as light yellow with pasty consistency. The product had high acceptability and received favourable comments from the judges. It had balanced salt-acid-sweetness blend which indicate the satisfactory quantity of the ingredients used in the sauce preparation. The sauce had typical mushroom flavour though lactic and acetic acids, and the other ingredients used in sauce preparation also imparted their taste and aroma to the product. The original texture of unfermented mushroom was spongy and the use of lactic acid fermentation brought the consistency of mushroom sauce to the desirable level, in addition to improvement in flavour.

Table 2

Physico-chemical, microbiological and sensory quality characteristics of mushroom sauce

Characteristic	Mean ± S.D.
Total soluble solids	19.76 ± 0.25
Titratable acidity (% A.A.)	1.24 ± 0.05
pH	3.30 ± 0.04
Salt (% Sodium chloride)	4.25 ± 0.05
Colour (Tintometer units)	
Red	2.8 ± 0.06
Yellow	4.75 ± 0.17
Blue	2.6 ± 0.15
Relative viscosity (Flow)[a]	3.48 ± 0.16
Microbiological quality	
Total aerobic count (CFU g^{-1})	0.2×10^2
Anaerobic count	Nil.
Incubation at 37 °C	Negative
Sensory evaluation (On hedonic scale of 1 to 9)	
Attributes	Mean Score ± S.D.
Colour and appearance	7.18 ± 0.2
Consistency	6.74 ± 0.3
Taste	7.30 ± 0.2
Flavour	7.50 ± 0.35
Overall quality	7.72 ± 0.16

[a] Flow rate in min; S.D.: Standard deviation

3. Conclusions

– The results on dry salting fermentation of mushroom with or without blanching clearly demonstrate that it is not possible to ferment mushroom by this technique. Even addition of lactose did not stimulate the fermentation and led to the chemical and microbiological spoilage.

– Pure culture inoculation with lactic acid bacteria supported the fermentation in brined mushrooms with addition of lactose.

– *Lactobacillus plantarum* gave the highest acid production amongst the bacteria tried.

– The fermented mushrooms gave a sauce of acceptable physico-chemical, microbiological and sensory qualities and shelf life.

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ROLE OF PROTEASE FROM *PENICILLIUM ROQUEFORTI* IN THE MODIFICATION OF CHEESE SLURRY AND TRAPPIST CHEESE RIPENING

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An acid protease from *P. roqueforti* was used in modifying the cheese slurry which was added to milk for Trappist cheese. Addition of the protease to the cheese slurry induced a marked increase of pH 4.6 soluble and TCA-soluble nitrogen fractions during the 40 days of incubation. Protease unmodified and especially protease modified cheese slurry added to milk significantly induced an increase of soluble nitrogen fractions and accelerated the ripening of Trappist cheese. The use of this protease appears to be a potential method to accelerate the ripening of Trappist cheese.

Keywords: cheese, acceleration of ripening, protease

An extracellular acid protease isolated from *P. roqueforti* (type EC. 3.4.23) was characterized by ZEVACO and co-workers (1973) and its action and specificity in the ripening of cheese was reviewed in a few studies (GRIPON, 1987; LAW, 1987; CERNING et al., 1987).

The action of acid protease from *P. roqueforti* in an aseptic model curd was characterized by a large increase in pH 4.6-soluble nitrogen and non-protein nitrogen. It is characterized by wide specificity, but hydrolyzes short-chain peptides poorly and do not liberate amino acids. It was also pointed out that the acid protease had a fundamental role in the proteolysis induced by this microorganism during cheese ripening (GRIPON et al., 1977). The pH optimum of 5.5 for the hydrolysis of casein (MODLER et al., 1974) and the hydrolytic characteristics appear to be very suitable from the point of view of the potential use of this protease in improving quality and accelerating the cheese ripening.

The use of cheese slurries or pastes developed as a method of intensifying cheese flavour and accelerating cheese ripening was reported by a number of authors (LAW, 1978; FOX, 1988/89). Some commercial preparations of that type are available on the market and it was claimed to have a wide application in dairy products (CARDINALE, 1987).

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This study was undertaken to determine the role of acid protease from *P. roqueforti* in the degradation of protein in cheese slurry and to study effects of protease modified cheese slurry added to milk on the proteolysis and the acceleration of Trappist cheese ripening.

1. Materials and methods

1.1. Protease

The extracellular acid protease was isolated from *Penicillium roqueforti* strain S86. The culturing conditions, inoculum and medium used were described elsewhere (PETROVIC et al., 1990). The enzyme was isolated and partially purified by a modified procedure of MODLER and co-workers (1974), which was described elsewhere (PETROVIC et al., 1991). The unit of enzyme activity was defined as that amount of enzyme required to produce an increase in absorbance (Lowry-Folin) of 0.01 min^{-1} at 280 nm with a 1% solution of Hammersten casein at pH 5.75 as substrate (MODLER et al., 1974).

1.2. Trappist cheese

Trappist cheese was manufactured from pasteurized (72 °C, 20 s) milk standardized for 3.2% fat content to provide at least 45% fat in dry cheese matter. After warming the milk to 32 °C, the 1% (w/w) lactic acid starter culture, *Streptococcus lactis subsp. lactis* and *Str. lactis subsp. cremoris*, was added and incubated for 30 min. The milk was coagulated for 30–40 min after addition of commercial fluid rennet. The coagulum was cubed (1 cm each), the curd was allowed to separate (15 min), and scalded (39 °C) with stirring for 45 min. Settled curd was transferred to perforated hoops, drained, and pressed (10–12 h). The cheese was made in the standard wheel form (16 cm diameter × 6 cm height, approximate 1.3 kg). The cheese was salted in brine (18% NaCl, 4 h), dried (5 days at 16–18 °C), waxed and aged (10 °C) for 60 days. Samples for analysis were taken after salting and at 30 and 60 days of ripening.

Three trials of Trappist cheese in three variants each were made in this study: control cheese without cheese slurry added (T_1); cheese with control cheese slurry added (T_2); and cheese with protease modified cheese slurry added (T_3). Cheese slurries used in experiments were fermented for 30 days and they were applied at the level of 360 g per 100 l cheese milk as suggested by CARDINALE (1987). Before adding to the cheese milk a required amount of slurry was dispersed by vigorous agitation in 500 ml milk at 45 °C.

1.3. Trappist cheese slurry

Cheese slurries in three replicates were prepared from 1-day old green unsalted trappist normally-manufactured as described above. The procedure we adapted for preparing the slurries was suggested by RICHARDSON and NELSON (1968), and DULLEY (1976). The aimed composition of slurries were as suggested by CARDINALE (1987) for a basic specification of enzymatic modified cheese. Two types of slurries were prepared: control, without protease added and experimental, with protease added. Shredded curd was aseptically blended with 2% (w/w) NaCl and turned into a smooth semi-liquid paste form using a domestic model blender. The purified enzyme dissolved in 1 ml solvent was added to experimental slurries at the level of 6 units/100 g curd and thoroughly blended. Each lot of slurry was 500 g. The pastes were packed tightly into screw-capped jars after being covered with a paraffin wax and held at 20 °C for 40 days. Fermented slurry was blended with 3% (w/w) sodium dicarbonate and heat treated at 80 °C for 5 min, cooled and stored at 5 °C.

1.4. Analysis

The nitrogen content of cheese and various fractions were determined by the Kjeldahl method using a Kjeltec system I module, Tecator Hoganas, Sweden. Protein was calculated using the conversion factor 6.38. Soluble nitrogen (SN) and non-protein nitrogen (NPN) refer to the pH 4.6 soluble nitrogen and the 12% TCA (trichloracetic acid) soluble nitrogen fractions, respectively. Sharp's extraction solution was used in extracting the pH 4.6-soluble nitrogen fraction as described by KOSIKOWSKI (1966). The 12% TCA soluble nitrogen fraction was extracted from cheese using the procedure described by REVILLE and FOX (1978). The results are expressed as a percentage of total nitrogen.

The percentage of dry matter and NaCl were determined according to IDF Standard Methods, INTERNATIONAL STANDARD (1972), and (1982), respectively. Fat was measured by the Gerber method (YUGOSLAV STANDARD, 1976). The pH of samples was measured in a 50% cheese suspension prepared in distilled water using a pH meter (Iskra MA 5703). Acidity was determined by the titration of a 10-g cheese sample suspended in water with 0.25 mol l^{-1} NaOH and phenolphtalein indicator and reported as % lactic acid. Total count of bacteria was determined using a standard trypticase-yeast extract-glucose-agar (Plate count agar "Torlak") (YUGOSLAV STANDARD, 1980). Lactobacilli were determined using the method described by DE MAN and co-workers (1960), and lactic acid streptococci according to ELLIKER and co-workers (1956).

Statistical analysis of the results included a one-way ANOVA and linear regression analysis, using the "Microstat" statistical software package program by Ecosoft Inc. 1985 version.

2. Results

2.1. Cheese slurry

Cheese slurries prepared were in average composed of 49.87% dry matter, 26.75% fat, 18.95% protein, and 2% NaCl. The pH value was 5.25 and the titratable acidity was 0.56%. The quantity of major components was stable, but some changes in pH and acidity occurred during the 40 days of fermentation. The pH values were fluctuating between 5.25 and 5.30. The titratable acidity was gradually increasing from initial 0.56% to 2.14 and 2.36% by day 40 in control and experimental cheese mass, respectively. However, variation and difference between pH and acidity in control and experimental cheese slurry was not significant ($P > 0.05$), as revealed by the analysis of covariance. That indicates that these parameters were not affected by the protease added.

The results in Table 1 indicate an increase of SN and NPN during the fermentation of cheese slurry. For 40 days of fermentation the SN increased about 3.2 and 3.8 times in the control and experimental cheese slurry, respectively. However, the increase of NPN was considerably higher, 7.0 and 8.2 times both in the control and experimental samples. These results agree with the pattern of proteolysis resulting in a larger amount of NPN than SN reported in a study on the aseptic curd model with the same enzyme (GRIPON et al., 1977).

The generating rate of SN and NPN during fermentation was higher in the experimental cheese slurry with protease added. The difference between control and

Table 1

Change of soluble (SN) and non-protein (NPN) nitrogen during the fermentation of cheese slurry
(% of total nitrogen)

	SN				NPN			
Age	Control[a]		Experimental[b]		Control		Experimental	
(day)	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	12.85	1.99	12.85	1.99	5.28	0.58	5.28	0.58
3	16.72	1.70	21.96	2.76	14.19	2.36	19.58	2.78
10	18.66	1.26	24.89	1.09	16.37	1.36	21.37	1.62
20	23.99	2.69	32.37	1.47	19.15	0.82	25.08	0.78
30	30.65	2.55	40.54	1.94	24.56	2.86	36.49	3.93
40	41.56	3.76	49.06	1.66	37.07	6.77	45.00	2.85

[a] Cheese slurry without protease added; [b] Cheese slurry with protease added; S.D.: Standard deviation

Table 2

Regression analysis of soluble (SN) and non-protein (NPN) nitrogen contents during the ripening of cheese slurry and trappist cheese

Regression				S.e.	Degree of freedom	Probability
			Cheese slurry			
(1)	SN (C)[a]	=	12.472 + 0.670 D[f]	0.067	10	0.000002
(2)	SN (P)[b]	=	15.849 + 0.832 D	0.064	10	0.0000001
(3)	NPN (C)	=	8.073 + 0.643 D	0.100	10	0.00008
(4)	NPN (P)	=	10.742 + 0.849 D	0.102	10	0.00001
			Trappist cheese			
(5)	SN (T_1)[c]	=	9.479 + 0.264 D	0.036	7	0.00015
(6)	SN (T_2)[d]	=	11.931 + 0.257 D	0.033	7	0.00011
(7)	SN (T_3)[e]	=	15.891 + 0.227 D	0.030	7	0.00013
(8)	NPN (T_1)	=	8.917 + 0.184 D	0.028	7	0.00035
(9)	NPN (T_2)	=	10.692 + 0.202 D	0.034	7	0.00054
(10)	NPN (T_3)	=	11.984 + 0.236 D	0.035	7	0.00028

S.e.: Standard error of regression coefficient; [a]C: Control cheese slurry (CS); [b]P: Protease modified cheese slurry (PMS); [c]T_1: Control trappist cheese; [d]T_2: Trappist cheese with CS added; [e]T_3: Trappist cheese with PMS added; [f]D: Day, age of cheese

experimental cheese slurry was found to be highly significant, $P < 0.001$ and $P < 0.01$ for SN and NPN, respectively. That indicates that the addition of protease accelerated the degradation of protein in cheese slurry. Veal and fungal rennets in Colby cheese slurries exhibited an increase in soluble protein during the 3-day fermentation period (RICHARDSON & NELSON, 1968). That increase appears to be less than we recorded with acid protease.

The SN to NPN ratio during the period of fermentation was quite stable. Consequently, a very high correlation coefficient was found between SN and NPN, $R = 0.97$ and 0.98 for the control and experimental cheese slurry, respectively.

Linear regressions fitted the best for the experimental data of SN and NPN in cheese slurries during the 40 days of fermentation (Table 2, equations 1–4). The probability values indicate that the estimates by these equations are highly reliable. The rate of proteolysis, as measured by the level of SN and NPN, can be calculated by these regressions. The regression coefficients indicate the higher rate of proteolysis in cheese slurry with protease added. Also, it can be calculated that the level of SN and NPN reached after 30 days of fermentation in cheese slurry without added protease can be achieved in about 20 days in cheese slurry with protease addition. The accelerated proteolysis reduced the fermentation time for about 33%.

Table 3

Chemical properties of Trappist cheese

	Age (day)					
	1		30		60	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
			Control cheese			
Dry matter, %	50.79	0.73	54.53	3.13	60.76	2.69
Fat, %	27.25	0.25	27.00	2.00	30.50	1.50
Protein, %	22.39	1.02	22.71	3.25	26.63	1.76
NaCl, %	1.27	0.02	1.36	0.03	1.45	0.05
pH	5.39	0.20	5.55	0.07	5.85	0.17
Acidity, %	1.07	0.13	1.71	0.45	1.98	0.18
			Cheese with control cheese slurry			
Dry matter, %	47.52	2.51	54.16	1.17	59.01	0.17
Fat, %	26.25	1.25	27.50	1.00	29.75	0.25
Protein, %	21.47	0.10	23.45	2.00	25.58	0.71
NaCl, %	1.09	0.12	1.26	0.07	1.34	0.10
pH	5.46	0.15	5.27	0.15	5.66	0.15
Acidity, %	1.04	0.23	2.11	0.01	2.02	0.32
			Cheese with protease modified cheese slurry			
Dry matter, %	50.56	0.96	54.56	2.54	59.77	1.28
Fat, %	26.00	1.00	27.50	1.50	30.25	0.75
Protein, %	21.38	1.60	22.78	0.64	25.99	0.46
NaCl, %	1.33	0.05	1.37	0.06	1.46	0.06
pH	5.43	0.10	5.30	0.08	5.56	0.16
Acidity, %	1.25	0.01	2.06	0.17	1.94	0.40

S.D.: Standard deviation

2.2. Trappist cheese

The contents of macrocomponents in Trappist cheese during ripening and due to the experimental treatments are given in Table 3. The slight differences occurring among control and experimental cheeses were not significant ($P > 0.05$). Also, differences in pH values and titratable acidity among control and cheeses with cheese slurry added were not significant ($P > 0.05$). The results indicate that pH and titratable acidity were not affected by adding either control or protease modified cheese slurry.

The results in Table 4 show the contents of SN and NPN during the ripening of Trappist cheese due to the experimental treatment. The initial contents of SN and

NPN were affected by the addition of cheese slurry. The highest content of SN was 16.58% in cheese with protease modified cheese slurry compared to the 11.25 and 13.01%, measured in the control cheese and cheese with protease unmodified cheese slurry, respectively. Also, the highest content of NPN (12.52%) was found in cheese with protease modified cheese slurry compared to the control cheese and cheese with protease unmodified cheese slurry, 10.36 and 10.91%, respectively. The increase of SN and NPN during the 60 days of storage can be expressed by linear regressions shown in Table 2 (equations 5–10). The standard error of regression coefficient and probability values indicate very good fitting of linear regressions to the experimental data. The experimental data (Table 4) and the slope of the curves (Table 2, equations 5–10) indicate that the generating rate of SN and NPN during storage is not exactly the same in control and experimental cheeses, but they are very close, between 0.23 and 0.26 for SN and 0.18 and 0.24 for NPN. This analysis indicates that the accelerated breakdown of cheese proteins due to the cheese slurry added did not occur during storage. The level of SN and NPN fractions depended highly on their initial content. Therefore, the level of these fractions at any time of storage may be estimated by the following

$$S_t = S_0 + 0.245\ t$$

and

$$N_t = N_0 + 0.204\ t$$

where S_t is soluble nitrogen and N_t is non-protein nitrogen (% of total nitrogen) on the day of storage t; S_0 and N_0 are initial percentages of soluble and non-protein nitrogen.

Table 4

Soluble (SN) and non-protein (NPN) nitrogen in Trappist cheese (% of total nitrogen)

Trial	Age (day)		
	1	30	60
		Soluble nitrogen	
T_1	11.25 (0.69)^a	14.43 (0.12)^a	26.78 (1.78)^a
T_2	13.01 (0.21)^a	16.44 (1.05)^b	28.17 (0.80)^a
T_3	16.58 (1.48)^b	20.03 (1.67)^c	29.97 (0.66)^b
		Non-protein-nitrogen	
T_1	10.36 (0.54)^a	11.94 (0.71)^a	21.16 (0.32)^a
T_2	10.91 (0.71)^a	14.40 (2.00)^{ab}	22.84 (0.16)^b
T_3	12.52 (1.97)^a	16.63 (1.87)^{bc}	26.44 (2.10)^c

T_1, T_2 and T_3 as in Table 2; ^{a,b,c} Means for soluble and non-protein nitrogen followed by different superscript letters differ significantly ($P < 0.05$)

Table 5

Bacterial population of trappist cheese during the 60 days of ripening

Cheese	Mean (log_{10} cfu g^{-1})	Standard deviation
	Total count	
T_1	7.01[a]	0.55
T_2	7.46[ab]	1.06
T_3	7.61[bc]	0.61
	Streptococci	
T_1	5.02[a]	0.96
T_2	5.23[a]	1.34
T_3	5.97[a]	1.09
	Lactobacilli	
T_1	6.23[a]	1.00
T_2	6.72[a]	0.39
T_3	6.83[a]	0.42

T_1, T_2 and T_3 as in Table 2. [a,b,c] Means for same bacterial group followed by different superscript letters differ significantly ($P < 0.05$)

These regressions can be used in estimating SN and NPN at any time of storage with very high reliability. Standard error of regressions for SN and NPN was 0.025 and 0.024, respectively. For these regressions T-test values were 9.74 and 8.66, and at the degree of freedom 25, they were found to be highly significant ($P < 0.001$). Applying these equations it can be calculated that the level of SN and NPN obtained during 30 days of ripening in control cheese can be achieved in cheese with protease modified cheese slurry and in cheese with control cheese slurry in about 8 and 19, and in 23 and 27 days, respectively.

Results shown in Table 5 indicate that the slurries added to cheese induced an increase in total count of bacteria, streptococci and lactobacilli. However, the mean values of streptococci and lactobacilli in experimental cheeses were significantly different neither from the control nor from cheeses with different slurries at $P < 0.05$. The only significant difference was found in total counts between control and cheese with protease modified slurry added.

3. Conclusions

The SN and NPN were gradually increasing during the 40 days of fermentation. The increase in NPN level was considerably higher compared with the amount of SN.

These results agree with the pattern of proteolysis resulting in a larger amount of NPN than SN reported in a study on aseptic model of curds with the same enzyme (GRIPON et al., 1977).

The addition of protease accelerated the degradation of protein in the cheese slurry. RICHARDSON and NELSON (1968) found that veal and fungal rennets in Colby cheese slurries exhibited an increase in soluble protein during the 3-day fermentation period. But, that increase appears to be less than we recorded with acid protease.

The protease added to cheese slurry improved specific cheese flavour. The cheese flavour developed was more intensive in 3-day old cheese slurry with protease added than in control slurry.

Bitter flavour as it was reported by RICHARDSON and NELSON (1968) when veal and fungal rennets were used in Colby cheese slurries was not detected. After 10 days of incubation the bitter flavour was not yet detectable as it was developed in some Cheddar cheese slurries with various animal and microbial protease combinations observed by KOSIKOWSKI and WASAKI (1975). However, a very mild bitterness was detected in experimental slurries by 20 days of incubation, but not in control slurries by 30 days. During 40 days of incubation the experimental slurries were characterized by more intensive cheese flavour than control slurries. A yellowish colour of experimental slurries was observed by 3 days of incubation, but that colour was developed in control slurries by 20 days.

The use of a protease modified slurry in Trappist cheese accelerated in the first place the primary breakdown of proteins and the formation of pH 4.6 soluble nitrogen fractions rather than the secondary products such as non-protein fractions. The accelerated proteolysis reduced the time required for the formation of soluble and non-protein nitrogen fractions for about 73 and 36%, respectively. In contrast with these results and other acceleration methods, the use of slurry in Cheddar cheese did not appear to increase proteolysis (TCA-soluble nitrogen), but accelerated ripening was attributed to an increased number of lactobacilli ($\log_{10}$ 5–7 cfu g^{-1}) (DULLEY, 1976). BOCKELMANN and LODIN (1974) also reported that increasing the number of lactobacilli from $\log_{10}$ 6–9 cfu g^{-1} by adding ripened cheese slurries to milk for Swedish Prästost accelerated markedly the ripening process.

Results shown in Table 5 indicate that the slurries added to cheese induced an increase in total count of bacteria, streptococci and lactobacilli. However, the mean values of streptococci and lactobacilli in experimental cheeses were significantly different neither from control nor from cheeses with different slurries at $P < 0.05$. The only significant difference was found in total counts between control and cheese with protease modified slurry added.

Addition of Trappist cheese slurry, especially slurry modified by acid protease from *P. roqueforti* appears to be a potential method to accelerate the ripening of

Trappist cheese. The problem of residual enzyme activity in ripened cheese during storage and distribution pointed out by GRIPON and co-workers (1977) was overcome in this study by the application of heat treated slurry.

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UTILIZATION OF PUMPKIN SEED AND RAPESEED PROTEINS IN THE PREPARATION OF BOLOGNA TYPE SAUSAGES

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Bologna type sausages containing 3% pumpkin seed (meal, protein concentrate and protein isolate) and rapeseed (protein concentrate and protein isolate) were prepared and compared with control. These additions did not appreciably affect viscosity, pH, crude protein content, fat content and amino acid composition. The colour of sausages was not changed by these ingredients except RPC-sausages which had lower lightness and redness values as compared to the control and the other treatments. Water holding capacity, cooking yield, moisture and ash contents of sausages were slightly affected by these additions. Content of some minerals increased (P, Cu, Fe, Mn and Mg) and other decreased (Na, Ca, K and Zn). Appearance and the texture in the products prepared with these ingredients were not significantly different. However, flavour and taste of control were significantly better than those prepared with rapeseed products but not significantly different from those prepared with pumpkin seed products.

Keywords: pumpkin seed, rapeseed, sausages, physical and sensory properties

Vegetable proteins are widely utilized as binders and extenders in meat systems to enhance particular functional properties and reduce costs. Intensive studies were carried out with various protein additives in meat products, such as soy flour concentrate and isolate (MILES et al., 1984), sunflower proteins (WILLS & KABIRULLAH, 1981) rapeseed protein concentrate (THOMPSON et al., 1982) and corn germ protein flour (BROWN & ZAYAS, 1990).

MANSOUR and co-workers (1993a) prepared rapeseed protein isolate by extraction the meal at pH 12.1, followed by single-step precipitation at pH 3.2. This protein isolate had a light black-brown colour with nitrogen yield of 68.3%. When 0.1% sodium metabisulfite was added to the clear supernatant and soaked the precipitate in 0.2% sodiummetabisulfite for 1 h followed by washing with water, a lighter coloured (light beige) isolate was obtained with a nitrogen yield of 63.6%.

MANSOUR and co-workers (1993a, b) concluded that pumpkin seed and rapeseed proteins have good functional properties that would allow it to be utilized in

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meat products. THOMPSON and co-workers (1982) extended ground beef patties with 3% of rapeseed protein concentrate and found that extended patties did not differ significantly from patties only from beef in appearance and texture, but the flavour was less acceptable.

There are no report on the use of pumpkin seed proteins in meat products. Also, very limited information is available on the use of rapeseed proteins in this respect. Therefore, our objective was to evaluate the effect of pumpkin seed and rapeseed proteins on physical and sensory properties, chemical composition, amino acid profile and mineral content of Bologna-type sausages ("Pariser sausages").

1. Materials and methods

1.1. Oilseed proteins

1.1.1. Pumpkin products. Pumpkin meal (PM), pumpkin protein concentrate (PPC) and pumpkin protein isolate (PPI) were prepared according to MANSOUR and co-workers (1993a). Details on the composition of the products are given in Table 1.

1.1.2. Rapeseed products. Rapeseed protein concentrate (RPC) and rapeseed protein isolate (RPI) were prepared according to MANSOUR and co-workers (1993c).

1.2. Bologna type sausage ("Pariser") formulation

The basic formulation of the "Pariser" was 55% lean beef, 20% beef tallow, 20% water, 2% salt, 1% dextrose, 1.5% spice mixture, 0.3% sodium tripolyphosphate and 0.2% pickling mixture. The oilseed proteins (PM, PPC, PPI, RPC and RPI) were added at a level of 3% related to the weight of beef lean, in powder form.

Table 1

Proximate composition of protein concentrates and isolates from pumpkin seed and rapeseed (on the dry weight basis, g per 100 g)

Product	Crude protein	Crude fat	Crude fibre	Ash	Carbohydrate
Pumpkin seed					
Meal	72.1	4.3	2.6	9.4	9.1
PPC	76.7	2.2	4.0	9.9	6.7
PPI	96.3	1.7	0.0	2.7	0.3
Rapeseed					
RPC	89.4	0.2	0.1	7.8	1.1
RPI	92.2	0.4	0.1	3.8	1.6

1.3. Preparation of sausages

Beef lean, salt, pickling mixture and 1/3 of the total added water were chopped in a cutter for 1–2 min before addition of the spice mixture. The beef tallow and another 1/3 of the total water was then added and chopping was continued for 3 min. The dry oilseed proteins and the final 1/3 of the total water were added. Chopping was continued for about 7 min until the sausage mix temperature reached 20 °C.

The sausage mix was then stuffed into synthetic casing (6.5 cm diameter and 40 cm length). Triplicates were prepared from each treatment. The sausages were cooked in water at 72 °C for 40 min, without smoking. After chilling in tap-water to about 15 °C, they were kept in refrigerator (5 °C) for one week prior to chemical, physical and sensory analysis.

1.4. Water holding capacity (WHC)

The modified Hamm procedure (HAMM, 1960) was used to measure the water holding capacity of the raw sausage mix. Sausage mix (0.3 g) was placed on filter paper (Whatman No. 1, stored overnight in saturated KCl) which was placed between two glass sheets and pressed for 20 min by 1 kg weight. The areas were measured with a compensating polar planimeter and the WHC was calculated by the following equation:

$$\text{WHC} = \left(1 - \frac{\text{Total area-Meat film area}}{\text{Meat film area}}\right) \times 100$$

1.5. Viscosity

Viscosity measurement was made on the sausage mixed at 20 °C using Rheotest-2 viscometer with a standard spindle rotating at 12 r.p.m. The readings were noted after 30 s shearing time and recorded in centipoise. Five replicates were measured for each treatment.

1.6. pH value

The pH values were determined using a 10% suspension (w/v) of the sausage mix in water by a Radelkis pH meter (Type, PO-264/1, Hungary).

1.7. Cooking yield

Cooking yield was determined by the weight difference between the raw and cooked products. Triplicate determinations were made for each treatment.

1.8. Colour evaluation

Tristimulus colour coordinates (L*, a*, b*) were measured on sausages using a reflectance photometer Momcolor Dc, measure head for fluids. Sausages were cut in half, and peeled slices (40 mm) were used for colour measurement. Five replicates were made for each treatment. The CIE values (L*, a*, b*) were used to calculate the hue angle and saturation index according to LITTLE (1975) as follows:

$$\text{Hue angle} = \tan^{-1} b^*/a^*$$
$$\text{Saturation index} = (a^{*2}+b^{*2})^{1/2}$$

1.9. Analytical methods

Moisture content (No. 14.004), crude protein content (N×6.25, No. 2.057), lipid content (No. 7.056) and total ash content (No. 14006) were determined according to the methods of A.O.A.C. (1980).

Amino acids were determined using a Mikrotechna AAA 881 automatic amino acid analyzer according to MOORE and STEIN (1963). Protein hydrolysis was performed in the presence of 6 mol HCl at 110 °C for 24 h in nitrogen atmosphere. Sulphur containing amino acids were determined after performic acid oxidation. Tryptophan was determined by the method of MILLER (1967).

Chemical scores of amino acids were calculated using the FAO/WHO (1973) reference pattern. Essential amino acid index (EAAI) was calculated according to OSER (1959) using the amino acid composition of freeze-dried whole egg protein as reference.

Phosphorus content was assayed photometrically via the phosphorus molybdate complex (FISKE & SUBBAROW, 1925). Minerals were determined according to the method of LINDNER and DWORSCHÁK (1966), either by wet ashing with nitric acid (Na, K, Ca) of by dry ashing (Cu, Zn, Fe, Mn, Mg) using a flame photometer (Flamom) and a Perkin Elmer (Model 403) atomic absorption spectrophotometer, respectively.

1.10. Sensory properties

Sausages were cut into slices and served to panel at room temperature. Six trained panelists evaluated the products for appearance, flavour, taste and texture using a 5-point hedonic scale: 5 = excellent, 4 = good, 3 = satisfactory, 2 = acceptable and 1 = nonacceptable.

1.11. Statistical analysis

Physical and sensory properties, colour and chemical composition data were evaluated by analysis of variance using the Statistical Analysis System (SAS, 1985). Differences at 5% level were considered as significant.

2. Results and discussion

Table 2 shows the WHC, viscosity and pH value of sausage mixes and cooking yield of the end products. The mean WHC between control and pumpkin seed containing sausages were not significantly different ($P > 0.05$). However, sausages with rapeseed products were significantly higher ($P < 0.05$) in WHC than control and those containing pumpkin seed products. These results are in agreement with those of ZAYAS and LIN (1989) and BROWN and ZAYAS (1990).

There were no significant differences ($P > 0.05$) in the viscosity of raw sausages mixed between control and other experimental products. These results agree well with those reported by LIN and ZAYAS (1987) and ZAYAS and LIN (1989). They found that the viscosity of sausage mixes was not changed by addition of 3% corn germ protein.

No significant differences ($P > 0.05$) could be observed in pH among the sausage mixes. BROWN and ZAYAS (1990) found that the pH of raw beef patties did not differ from those prepared with 10% corn germ protein flour slurry. Similar observations were reported by TROUTT and co-workers (1992) for ground beef containing pea fiber, potato starch and oat fiber.

The cooking yield of sausages prepared with oilseed products was significantly higher ($P < 0.05$) than that of the control. Sausages prepared with pumpkin seed products were not significantly different ($P > 0.05$) in cooking yield compared with

Table 2

Physical properties of Bologna type sausage mixes and end-products

Treatment	WHC (%)		Viscosity $\times 10^5$ (cPs)		pH		Cooking yield (%)	
	x	s	x	s	x	s	x	s
Control	85.3a	1.7	3.2a	0.1	5.9a	0.2	84.3a	1.8
				Pumpkin				
Meal	87.8a	1.5	3.6a	0.1	6.0a	0.2	97.6b	1.7
Concentrate	87.9a	1.8	3.3a	0.1	6.0a	0.2	98.7b	1.7
Isolate	87.1a	1.2	3.4a	0.1	5.8a	0.1	98.8b	1.9
				Rapeseed				
Concentrate	97.4b	1.8	3.5a	0.2	5.9a	0.1	96.5b	0.9
Isolate	89.5b	1.6	3.6a	0.2	5.8a	0.1	98.5b	1.0

x: mean of triplicates; s: standard deviation. The same subscript in the same column means that the difference is not significant at 5% probability level

the sausages prepared with rapeseed. These results are consistent with those reported by LIN and ZAYAS (1987), ZAYAS and LIN (1989), BROWN and ZAYAS (1990) and MILES and co-workers (1984). They found that the cooking yield of mixes from only beef was significantly lower than the cooking yield of products prepared with 7% soy flour or 5.5% soy concentrate.

Results of colour evaluation are presented in Table 3. Sausages prepared with RPC were significantly darker (L* value) and significantly lower in redness (a* value) than the control and other experimental treatments. Addition of rapeseed resulted in a higher hue angle (25.2) and a lower saturation index (24.8). However, there were not significant differences ($P > 0.05$) in the intensity of yellowness (b* value) among treatments. These results indicate no appreciable colour changes in sausages prepared with the addition of pumpkin seed products and RPI. However, the colour changes in sausages prepared with RPC might be attributed to the presence of polyphenols which were oxidized to greyish-brown colour during processing.

These results agree well with those reported by BROWN and ZAYAS (1990) for broiled beef patties prepared with 10% corn germ slurry by LIN and ZAYAS, (1987) for sausages prepared with 4% corn germ. ZAYAS and LIN (1989) found that the colour of sausages did not change by the addition of 3% corn germ protein.

Evaluation of sensory properties is shown in Table 4. No significant differences ($P > 0.05$) in texture and appearance were found among treatments. Flavour and taste were not significantly different ($P > 0.05$) between control and those with pumpkin products. However, sausages prepared with rapeseed products were significantly lower ($P < 0.05$) in flavour and taste than control. ZIPRIN and co-workers (1981) found that beef patties prepared with 3.3% soy or peanut (flour, concentrate or isolate) were not significantly different in flavour, juiciness and texture.

The proximate composition of Bologna type sausages is presented in Table 5. There was no significant difference ($P > 0.05$) in moisture content between control and sausages prepared with RPI. However, moisture content in sausages prepared with pumpkin products was, as expected, significantly lower ($P < 0.05$) than the control and those prepared with rapeseed products. Protein and fat content in all sausages were not significantly different. Further experiments are needed to explain the lack of expected significant differences after the addition of the powdered protein products.

Data in Table 6 show that amino acid composition in sausages was not affected by the addition of oilseed proteins with the exception of lysine and tyrosine. Frankfurters substituted with PPI, RPI, RPC had much higher lysine content than the control. However, lysine content in control was higher than those prepared with PM and PPC. The concentration of tyrosine in control was higher than those prepared with rapeseed products, but did not differ from those prepared with pumpkin products. The results are in accordance with the earlier work of MANSOUR and co-workers (1993a) reporting the high lysine and low tyrosine level of rapeseed protein.

Table 3

Colour evaluation of sausages

Treatment	CIE values						Hue angle		Saturation	
	L*		a*		b*					
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Control	61.7a	1.3	26.7a	0.5	10.3a	0.1	21.0a	0.1	28.7a	0.2
					Pumpkin					
Meal	62.3	1.3	26.8a	0.2	10.4a	0.2	21.3a	0.3	28.7a	0.4
Concentrate	63.3a	1.1	26.2a	0.1	11.0a	0.1	22.8a	0.4	28.5a	0.5
Isolate	63.0a	1.5	27.0a	0.4	10.3a	0.1	20.9a	0.2	28.9a	0.3
					Rapeseed					
Concentrate	59.3a	0.9	22.4b	0.6	10.6a	0.2	25.2b	0.4	24.8b	0.3
Isolate	62.4a	1.6	26.0a	0.7	11.0a	0.1	24.1ab	0.5	28.4a	0.3

$\bar{x}$: mean value of three determinations; s: standard deviation. The same subscript in the same column means that the difference is not significant at the 5% probability level

Table 4

Sensory evaluation of sausages

Treatment	Appearance		Flavour		Taste		Texture	
	x	s	x	s	x	s	x	s
Control	3.4a	0.3	4.2a	0.2	4.2a	0.2	3.8a	0.1
				Pumpkin				
Meal (PM)	3.6a	0.2	4.0ab	0.2	4.0ab	0.1	4.2a	0.2
Concentrate (PC)	3.2a	0.1	3.6ab	0.2	3.6ab	0.1	4.0a	0.2
Isolate (PI)	3.4a	0.1	3.6ab	0.1	3.6ab	0.2	3.8a	0.1
				Rapeseed				
Concentrate (RPC)	3.6a	0.2	3.4b	0.1	3.4b	0.1	3.4a	0.2
Isolate (RPI)	3.6a	0.1	3.4b	0.2	3.4b	0.1	3.4a	0.2

x: mean value of three determinations; s: standard deviation. The same subscript in the same column means that the difference is not significant at the 5% probability level

Table 5

Proximate composition of Bologna-type sausages
(on dry weight basis in %)

Treatment	Moisture		Crude protein		Crude fat		Ash	
	x	s	x	s	x	s	x	s
Control	57.2c	1.0	35.3a	1.2	57.5a	1.4	5.4a	0.2
				Pumpkin				
Meal	52.6a	0.9	35.9a	1.1	57.6a	1.2	7.1b	0.3
Concentrate	53.7a	1.1	36.5a	0.8	56.4a	1.3	7.3b	0.3
Isolate	52.9a	0.8	38.9a	0.9	57.2a	1.1	5.3a	0.1
				Rapeseed				
Concentrate	55.4b	1.0	37.5a	1.3	56.7a	1.5	6.2ab	0.2
Isolate	56.0c	1.1b	38.0a	0.8	56.8a	1.3	6.0ab	0.1

x: mean value of three determinations; s: standard deviation. The same subscript in the same column means that the difference is not significant at the 5% probability level

The chemical score of control was 55% similar to those substituted with PPC and PPI.

However, sausages substituted with PM, RPC and RPI had higher chemical scores than control and other products probably owing to their high lysine content. Sulphur containing amino acids and valine were the first and second limiting amino acids respectively in all frankfurters.

Table 6

Amino acid composition (g/16 g N), *chemical score and essential amino acid index* (EAAT) *of sausages*

Amino acids	Control	Pumpkin			Rapeseed	
		Meal	Concentrate	Isolate	Concentrate	Isolate
Isoleucine	3.6	3.5	3.3	3.9	4.1	3.4
Leucine	7.0	6.9	6.8	7.1	7.0	7.3
Lysine	7.1	6.1	5.7	9.0	10.6	8.5
Cystine	0.7	0.7	0.8	0.8	0.9	1.1
Methionine	1.5	1.7	1.3	1.6	1.8	1.8
Total sulphur amino acids	2.2	2.4	2.1	2.4	2.7	2.9
Tyrosine	4.0	4.3	3.3	3.9	2.7	3.1
Phenylalanine	4.5	4.9	5.5	5.0	3.6	4.6
Total aromatic amino acids	8.5	9.2	8.8	8.9	6.3	7.7
Threonine	6.1	5.1	5.3	6.2	6.7	5.7
Tryptophan	2.1	2.3	2.4	2.1	1.9	2.7
Valine	3.7	4.0	3.6	4.0	5.1	4.2
Total essential amino acids	40.3	39.5	38.0	43.6	44.4	42.4
Histidine	4.2	3.9	3.9	4.0	4.1	4.5
Arginine	6.4	9.2	7.8	8.0	5.7	6.3
Aspartic acid	11.3	11.8	12.1	11.3	10.3	10.1
Glutamic acid	13.1	12.2	12.4	11.6	15.4	12.9
Serine	4.8	5.1	7.4	4.7	2.4	5.2
Proline	6.0	5.3	4.7	4.7	5.8	5.4
Glycine	6.9	6.5	7.1	5.8	6.2	6.4
Alanine	7.0	6.5	6.6	6.3	5.7	6.8
Total non-essential amino acids	59.7	60.5	62.0	56.4	55.6	57.6
Chemical score %	55	61	55	55	61	68
First limiting amino acid	Met + Cys	Met + Cys	Met + Cys	Met + Cys	Met + Cys	Met + Cys
Second limiting amino acid	Val	Val	Val	Val	Val	Val
EAAI %	77	77.5	75.5	79.8	79.7	80.2

Table 7

Mineral content of sausages (on dry weight basis in mg per 100 g)

Treatment	Na	Ca	K	P	Cu	Zn	Fe	Mn	Mg
Control	1985.9	88.8	420.7	267.1	0.2	16.4	4.0	0.2	151.9
Pumpkin meal	1582.2	82.3	407.2	273.6	0.3	11.9	5.5	0.8	200.4
Pumpkin protein concentrate	1641.5	88.6	438.4	281.6	0.3	15.1	6.3	0.9	180.4
Pumpkin protein isolate	1719.7	76.4	371.5	248.8	0.4	0.9	4.7	0.2	90.2
Rapeseed protein concentrate	1860.9	109.9	372.2	339.4	0.2	10.1	5.3	0.4	160.2
Rapeseed protein isolate	1818.2	106.8	418.2	311.6	0.2	11.1	5.0	0.6	125.0

The EAAI of frankfurters ranged between 77–80%. There was a slight improvement in EAAI by the addition of oilseed proteins to the sausages except those prepared with PPC. These values are in good agreement with the biological value of meat (74%) and fish (76%) reported by KANWAR and co-workers (1991).

Data in Table 7 show that the content of P, Cu, Fe, Mn and Mg increased, while Na, Ca, K and Zn contents decreased with the addition of oilseed proteins to the sausages. Sausages prepared with PPI had a lower mineral content except Cu as compared to the other products. Higher Cu content in PPI-sausages can be attributed to the high Cu content in PPI (MANSOUR et al., 1993b). Bologna-type sausage prepared with PPC was higher in micro elements and K than that prepared with RPC. On the other hand sausage prepared with RPC had higher Na, Ca and P content than that of PPC-product sausage. Generally, the mineral content of these sausages varied considerably depending on the mineral content of oilseed proteins (MANSOUR et al., 1993c).

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EFFECT OF FABA BEAN TANNINS ON NUTRIENT ABSORPTION IN THE SMALL INTESTINE OF RAT

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The aim of the work was to study the effect of tannin rich extract of phenolics from faba bean seed hulls on glucose and methionine absorption from the small intestine of rats. The extract obtained by extraction of faba bean seed hulls with 70% aqueous acetone contained, after lyophylization, 49.5% proanthocyanidins. The presence of proanthocyanidin oligomers was confirmed chromatographically. In biological experiments, the effect of the extract obtained and of chemically pure tannins on the absorption of glucose, methionine and water in the small intestine of rat was determined using gut perfusion and everted sacs method. Proanthocyanidins from the Nadwislanski faba bean seed hulls administered to the rat gut in the amount corresponding to the content of these compounds in daily diet with 20–40% faba beans content had no significant effect on glucose or methionine absorption but they increased water absorption. Significant lowering of glucose and methionine absorption took place when perfusion fluid was supplemented with analogical amount of chemically pure tannins.

Keywords: tannins, faba bean hulls, phenolic compounds, glucose, methionine, rat intestine absorption

The seed hull of faba beans is the site of condensed tannins considered to be an important antinutritional factor of this legume species (MARQUARDT et al., 1977). By forming complexes with diet proteins, digestive enzymes and the endothelium of digestive tract tannins reduce digestibility and absorbability of nutrients in the alimentary tract of monogastrics (JANSMAN et al., 1994; LONGSTAFF & MC NAB, 1991). This was showed in experiments in which animals were fed diets with high content of seed hulls or seed hull tannin extract (ALZUETA et al., 1992; JANSMAN et al., 1994; MITJAVILA et al., 1977; MOTILVA et al., 1983; ORTIZ et al., 1993; YUSTE et al., 1992). There are, however, no available data on experiments conducted on isolated animal guts with infusion fluids in which tannin concentration corresponds to the tannin quantity occurring in practical feeding of animals. The aim of the work was to study the effect of tannin rich extract of phenolics from faba bean seed hulls on glucose and methionine absorption from the small intestine of rats.

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1. Material and methods

1.1. Extraction

Cold extraction of phenolics from the ground seed hulls of Nadwislanski faba beans was conducted with 75% acetone. Solvent to raw material ratio was 3:1, v/m. After 24 h solvent was filtered and seed hulls were poured with acetone for another 6 h. Obtained extracts were combined, acetone was distilled out in a rotary evaporator, and water was removed by lyophilization.

1.2. Chemical analyses

Phenolic compounds were determined by two methods: as total polyphenols (NACZK & SHAHIDI, 1989) and as flavonols an proanthocyanidines (OSZMIANSKI et al., 1988). Separation of phenolics from the extract obtained was carried out in a column (1.5 cm × 80 cm) with Sephadex LH−20 using ethanol and methanol for elution. UV spectrum of the fractions obtained was determined on spectrophotometer Specord M−42. TLC analysis of fractions was made on plates Kisel gel 60 (Merck) applying *n*-butanol-water-acetic acid (3:1:1, v/v/v) developing system (ZADERNOWSKI, 1987). Phenolics were visualized on plate by spraying them with ferric chloride and ferrocyanide solution (BARTON et al., 1952), while tannins with vanillin solution in chloride acid (BATE-SMITH, 1953). Fraction washed out from the column with methanol was additionally separated on a plate with silica gel using chloroform-methanol-water (63:35:10, v/v/v/, lower layer) mobile phase (TANIZAWA et al., 1984), and on a plate with cellulose (Merck) by two-dimensional TLC technique applying t-butanol-water-acetic acid (3:1:1, v/v/v) (first developing system) and 6% acetic acid (second developing system) (FOO, 1984). Tannins were visualised by spraying plates with vanillin-chloric acid solution. 2−D LC was performed also for epi-catechin and epi-gallocatechin gallate standards.

1.3. In vitro *absorption*

The method of everted sacs (WILSON & WISEMAN, 1954) was used for determining *in vitro* gut absorption. Sections of the small intestine were everted the mucosa out and sacks, about 10 cm long and about 0.7 g, were made from them. The sacs were filled with 1 ml perfusion fluid and submerged in 10 ml intensively oxigenated incubation fluid of 37 °C. Thyrod fluid (NaCl − 8.045, KCl − 0.4, $CaCl_2$ − 0.279, KH_2PO_4 − 0.243, $MgSO_4$ − 0.215, $NaHCO_3$ − 0.375 g^{-1}) supplemented with glucose (2 g^{-1}) and methionine (1.491 g^{-1}) was used as initial perfusion and incubation fluid. In control (0) group fluid with identical composition, listed above, was administered on both sides of the gut wall. In experimental groups I and II the same perfusion fluid was administered into the sacs while outside them, i.e. on

the everted mucosa side, incubation fluid with addition of the tannin extract from faba bean seed hulls was applied 0.37 and 0.74 g^{-1}, respectively. The content of extract in perfusion fluid was so adjusted that an isolated section of the gut remained in contact with 1.83 or 3.66 mg proanthocyanidin which corresponded to the concentration of these compounds during the flow of daily diet (10 g) with 20% or 40% inclusion of faba beans through the gut. In group III, chemically pure tannin (Loba) was added (0.37 g^{-1}) to perfusion fluid instead of tannin extract from faba bean seed hulls. Samples of perfusion fluid were collected for analysis before and after incubation for glucose and methionine determination. Glucose content in perfusion fluids was determined enzymatically using diagnostic set Cormay GS – 120 while methionine by Stein-More's method acc. to TOMASZEWSKI (1970). The result of glucose and methionine absorption, determined in 23 sacs in every group, was expressed in mg of components absorbed by 1 g of intestine during 1 h. Water absorption (ml $g^{-1}h^{-1}$) was calculated from the difference in volume before and after incubation.

1.4. Absorption in situ

In situ technique in an open system, based on controlled flow of perfusion fluid through the small intestine of anaesthetised rats (FISHER & GARDNER, 1974), was used for determining gut absorption. The experiment was made on 104 Wistar rats divided into 4 groups, in each n = 26 animals of 210 – 240 g body weight. Animals were anaesthetised (subcutaneous administration of 10% sodium hexobarbiturate, 1 ml/100 g body weight) and placed on heated operating table. Following tracheotomy and laparotomy two drains were inserted in the small intestine: inlet right below the duodenum and outlet above the blind gut. Into thus limited section of the small gut, following washing with physiological saline sol (0.9% NaCl), perfusion fluid was administered. Regular, close to physiological rate of administration (1 ml min^{-1} was ensured by peristaltic pump PA – SK 8 by IKA-labortechnik (Janke Kunkel). Control rats (group 0) were given Thyrod fluid while experiment rats were given Thyrod fluid with addition of the substances studied: faba bean seed hull tannin extracts (groups I and II) or chemically pure tannin (group III). Perfusion proper lasted 15 min and was preceeded by 20 min introductory period. Results were expressed in mg glucose and methionine or ml water absorbed during 15 min perfusion.

In additional group of 12 rats an experiment was made successively with a long-term gut perfusion with 3 fluids: control Thyrod fluid (C), Thyrod fluid with addition 0.74 g l^{-1} of faba bean tannin extract (T), and again control fluid (C). Each fluid was administered preliminarily for 20 min and properly for 1 h. Perfusion fluid circulated in a closed circuit, and samples were collected every 15 min to determine glucose losses. During another 3 h of perfusion glucose absorption from control fluid and

fluid supplemented with faba bean tannin was determined. The amount of proanthocyanidines flowing through the gut during administration of perfusion fluid was 29.3 mg which corresponded to the amount of these compounds in daily diet of rats with 35% content of faba beans.

1.5. Statistical analysis

The data were statistically handled using variance analysis and Duncan's multiple range test.

2. Results and discussion

2.1. Chemical composition of acetone extracts

UV spectra of 4 fractions ($E_1 - E_4$) eluted from the column with ethanol were characterized by absorbance maxima at 280, 264, 264 and 280 nm (Fig. 1). UV spectrum of E_4 had an extra maximum at 330 nm resulting most likely from derivatives of phenolic acids. UV spectrum of fraction M, eluted from the column with methanol, had a maximum at 288 nm. Results of TLC analysis of the fractions

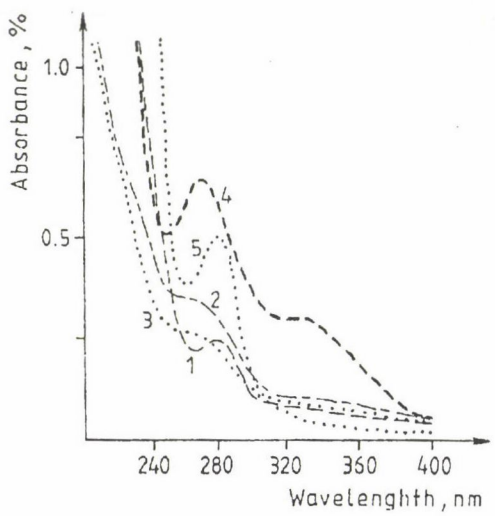

Fig. 1. UV spectra of the fraction from the column with Sephadex LH – 20. Fractions: 1: E_1; 2: E_2; 3: E_3; 4: E_4; 5: M

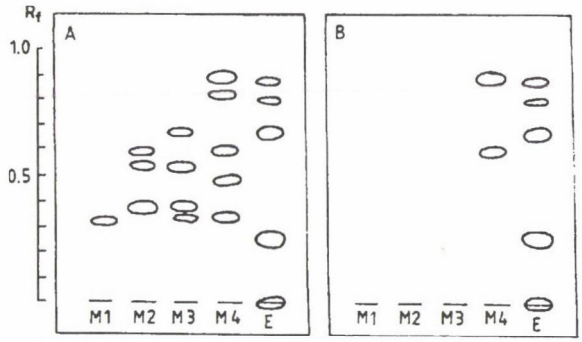

Fig. 2. TLC separations of the fraction from the column with Sephadex LH-20 – Mobile phase n-butanol-water-acetic acid = 3:1:1. A: spots produced by potassium ferricyanidine and ferric chloride; B: spots produced by vanillin

eluted from the column revealed the presence of several phenolics in each fraction (Fig. 2 and 3). The compounds giving red spots following spraying with vanillin occurred only in fractions E_4 and M. Values Rf of the compounds from fraction M giving positive reaction with vanillin were lower than Rf of epicatechine gallate. From the comparison of results of 2D-TLC with available literature data (KARCHESY et al., 1989) it follows that fraction M contained procyanidine oligomers, trimers and even larger particles. Total content of phenolics in the extract was 79.35%, and of proanthocyanidins 49.5% (Table 1). Similar content of condensed tannins (47.17%, expressed as catechin equivalents) in the extract from the faba bean seed hull was reported by ALZUETA and co-workers (1992) who applied extraction with 70% aqueous acetone. For the same extraction method ORTIZ and co-workers (1993) obtained extract containing 89.7% phenolics including 57% proanthocyanidins. On extracting seed hulls with water JANSMAN and co-workers (1994) obtained extract containing 21.8% condensed tannins expressed as catechin equivalents. The latter authors used the seed hulls of lower tannin content (5%) for their experiment. The Nadwislanski seed hulls analysed contained 6.53% proanthocyanidin (Table 1) which exceeded the amount reported by CANSFIELD and co-workers (1980) (2-6% of the seed hull) but was lower than that found by YUSTE and co-workers (1992) in the seed hulls of 3 faba bean varieties (7.4-9.7%). From the comparison with the literature data cited it follows that the seed hulls of Nadwislanski variety is characterized by a high tannin content.

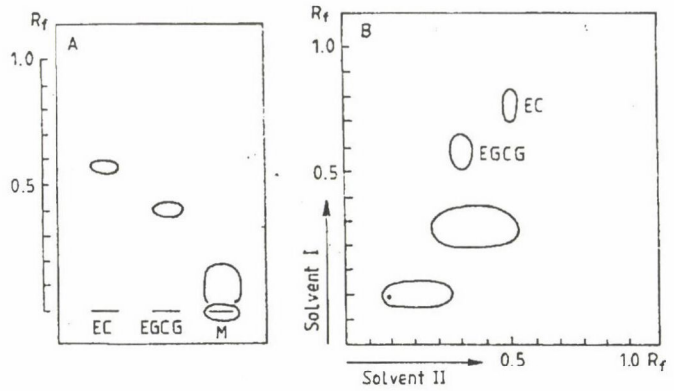

Fig. 3. TLC separations of the fraction washed out from the column with methanol. A: mobile phase; chloroform – methanol – water = 65:35:10; B: 2D-TLC; Solvent I: t-butanol – water – acetic acid = 3:1:1, Solvent II: 6% acetic acid

2.2. *Effect of tannins on* in vitro *gut absorption*

No statistically significant effect of the seed hull phenolics in perfusion fluid on glucose absorption by the intestine wall of rats was observed (Table 2). However, in group II, in which the amount of proanthocyanidins having contact with the mucosa of the isolated gut section (3.66 mg) corresponded to the concentration of these compounds in rats daily diet with 40% faba bean seeds content, some lowering of glucose absorption was observed, and it was similar to that found in group III following administration of perfusion fluid analogical addition (3.70 mg/sack) of pure tannins. Addition of pure tannins highly significantly reduced glucose absorption compared to control perfusion fluid and fluid with less addition of tannin extract (1.83 mg/sack) corresponding to the diet with 20% faba bean content. Methionine absorption was similar when control fluid with addition of tannin extract were administered, and highly significantly comparable when perfusion fluid was supplemented with pure tannins (Table 2).

Table 1

Phenolic compounds content in faba bean hulls and acetone extract (%)

Ingredient	Faba bean hulls		Acetone extract
	raw	extracted	
Total polyphenols	8.97	0.60	79.35
Proanthocyanidin	6.53	0.42	49.50
Flavanols	1.82	0.17	14.60

Table 2

Absorption of glucose, methionine and water from control (0) and perfusion fluid supplemented with faba bean tannins extract (I and II) or with pure tannin (III)[a]

Component absorbed[b]	Experiment group			
	0	I	II	III
Glucose [mg]	$1.12^{A} \pm 1.04$	$0.93^{A} \pm 0.49$	$0.81^{AB} \pm 0.60$	$0.76^{B} \pm 0.42$
Methionine [mg]	$2.77^{A} \pm 0.64$	$2.56^{A} \pm 0.64$	$2.45^{A} \pm 0.75$	$1.70^{B} \pm 0.53$
Water [ml]	$0.33^{A} \pm 0.16$	$0.39^{B} \pm 0.16$	$0.36^{AB} \pm 0.16$	$0.34^{A} \pm 0.16$

[a] Mean values; ± standard deviation for 32 sacks; [b] mg, ml l^{-1} g gut h^{-1} (in mg or ml per 1 g gut during 1 h of perfusion); [A,B] Means with the same superscripts within a column are not significantly different by Duncan's multiple range test at $P \leq 0.01$

2.3. *Effect of tannins in* in vitro *gut absorption*

Short (15 min) perfusion of the small intestine of gut with fluids supplemented with tannin extract from faba beans did not reduce glucose or methionine absorption compared to the absorption of these components from control perfusion fluid (Table 3). Highly significant inhibition of glucose absorption, both with regard to control group and groups with addition of faba bean tannin extract, was observed when perfusion fluid was supplemented with analogical addition of pure tannins. When perfusion time was elongated (Table 4) glucose absorption even increased following administration of perfusion fluid with addition of faba bean tannin extract. After another administration of control perfusion fluid glucose absorption returned to the initial value.

Table 3

Absorption of glucose, methionine and water from control (0) and perfusion fluid supplemented with faba bean tannins extract (I and II) or with pure tannin (III)[a]

Component absorbed[b]	Experiment group			
	0	I	II	III
Glucose [mg]	$14.06^{A} \pm 3.54$	$12.40^{A} \pm 3.07$	$12.05^{A} \pm 3.45$	$10.36^{B} \pm 3.47$
Methionine [mg]	$7.14^{A} \pm 1.92$	$6.71^{A} \pm 2.66$	$6.39^{A} \pm 1.70$	$5.35^{B} \pm 1.60$
Water [ml]	$1.46^{A} \pm 0.78$	$1.65^{B} \pm 0.94$	$1.64^{B} \pm 1.04$	$1.51^{AB} \pm 0.92$

[a] Mean values; ± standard deviation for 26 rats; [b] mg, ml/15 min perfusion (in mg or ml per 1 g gut during 15 min of perfusion); [A,B] Means with the same superscripts within a column are not significantly different by Duncan's multiple range test at $P \leq 0.01$

Table 4

Dynamics of glucose absorption from control (C) perfusion fluid and from fluid with faba bean tannins extract (TE)[a]

Perfusion fluid	Perfusion time (min)				
	0–15	16–30	31–45	46–60	0–60
C	$16.81^{B} \pm 1.48$	20.76 ± 3.66	$12.29^{B} \pm 1.36$	$10.31^{B} \pm 0.88$	$54.97^{B} \pm 2.29$
TE	$18.87^{A} \pm 0.69$	21.75 ± 3.13	$12.31^{AB} \pm 1.30$	$10.85^{A} \pm 0.77$	$58.49^{A} \pm 2.03$
C	$16.95^{AB} \pm 1.76$	21.04 ± 3.20	$13.16^{A} \pm 1.04$	$10.75^{AB} \pm 0.76$	$56.34^{B} \pm 2.80$

[a] mg (15 and 60 min perfusion/rat), mean values; ± standard deviation for 12 rats; ^{A,B} Means with the same superscripts within a column are not significantly different by Duncan's multiple range test at $P \leq 0.01$

Contrary to experiments by BARCINA and co-workers (1984) and MOTILVA and co-workers (1983) the extract of condensed faba bean tannins used in our experiment had no negative effect on glucose or methionine absorption in the small intestine of rats. This was most likely due to a lower amount of proanthocyanidins administered corresponding to that in the alimentary tract of rats following consumption of daily diet with 20 or 40% (*in vitro* experiment) or 30% (long-term gut perfusion) inclusion of faba beans. ORTIZ and co-workers (1993) reported highly significant lowering of total protein digestibility in chicks caused by addition of acetone extract corresponding to diet containing 100% faba beans. Also according to other authors (JANSMAN et al., 1994; YUSTE et al., 1992) inhibition of enzymes activity in the alimentary tract and significant lowering of nutrients digestibility was observed following introduction of high (30–40%) content of seed hulls or corresponding amount of condensed tannins. On applying lower addition of proanthocyanidins, corresponding to the content of these compounds in diets with 30–40% faba beans content (in this 5% of the seed hull) no significant changes in nutrients absorption by the gut wall of rats was observed.

3. Conclusion

The carried out analyses revealed that the seed hulls of faba bean Nadwislanski variety is characterized by the high tannin content, as proanthocyanidins (6.53%). Proanthocyanidins rich extract from seed hulls administered to the rat gut in the amount corresponding to the content of these compounds in daily diet with 20–40% faba beans content had no significant effect on glucose or methionine absorption but increased water absorption.

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ESTIMATION OF THE TURNOVER NUMBER OF LACCASE ENZYME

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The estimation of the enzyme kinetic parameters is always a challenging problem. This paper covers two methods to estimate the turnover number and illustrates these by the example of laccase enzyme. Both methods exploit the dynamic behaviour of the enzyme reaction, one of them uses the derivative values the other the integral ones.

The experimental system is characterized which can be described by the simple Michaelis-Menten enzyme reaction scheme. The results of this research are the estimation of the turnover number, the calculation of the total enzyme (which is active in this reaction) and the calibration coefficients between absorbance and molar concentration for the product. This calibration is called "calibration by kinetics".

Keywords: enzyme kinetics, turnover number, laccase, estimation of kinetic parameters

White-rot fungi (e.g. *Lentinus edodes*, *Pleurotus* and *Phanerochaete sp.*) play important role not only in feeding but because of their ability in lignin biodegradation, in recovery of the agricultural side products containing lignocellulose (HOSCHKE et al., 1988).

ANDER and ERICSSON (1977) pointed out that there is a relationship between the microbial phenoloxidase production and the lignin biodegradation. First time MATCHAM and co-workers (1985) produced lignolytic enzyme complex by fungi in fermentor. The enzyme mechanism of the lignin biodegradation was examined by KIRK and FARREL (1987). HAEMMERLI and co-workers (1986), HAMMEL and MOEN (1991), LEISOLA and co-workers (1988) studied the behaviour of the lignin peroxidase considered as the underlying component of the lignolytic enzyme system. MISHRA and LEATHAM (1990), ROGALSKI (1991), and ARCHIBALD (1992) made efforts to recover and fractionate the extracellular degradative enzymes from different cultivations.

The role of laccase (E.C. 1.10.3.2. O_2: diphenol oxidoreductase), out of the phenoloxidase enzymes operating in the lignin biodegradation, was investigated by

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BOURBANNAIS and PAICE (1990), and additionally MUCHEIM and co-workers (1992) pointed out that the laccase has an important part in the degradation. However polymerization reactions can occur during the lignin biodegradation because of the activity of laccase enzyme. Laccase is responsible for the demethylation and side-chain elimination of lignin and lignin-related model compounds (REINHAMMER, 1984). Moreover laccase has a considerable role in toxicity reduction of phenolic compound by polymerization reactions (BOLLAG et al., 1988).

In this paper we try to contribute to the description of the kinetic behaviour of the laccase enzyme.

1. Materials and methods

1.1. Enzyme-kinetics measurements

A modification of the laccase-enzyme activity measurement method of LEONOWICZ and GRZYWNOWICZ (1981) was applied. The method is based upon the measurement of the spectral change during the chromophore-producing reaction of the laccase enzyme and its artificial substrate syringaldazine.

Solutions:

Substrate: 0.018 g syringaldazine (4-hydroxi-3,5-dimetoxi-benzaldehide-azine) (SIGMA) ad 100 cm^3 96% ethanol solution.

Buffer: 100 cm^3 buffer solution contained 0.7836 g 2-(N-Morpholino)-ethane-sulphonic acid (MES) and 0.293 g EDTA.

The pH of solution was adjusted to 5.8 by NaOH.

Method:

A blank was prepared from 450 μl distilled water, 2.25 ml buffer solution and 300 μl substrate solution in a 4 cm^3 quartz-cuvette. The reaction mixture was prepared similarly in a 4 cm^3 quartz-cell containing 2.25 ml buffer and variable amount of substrate and enzyme solution giving a total volume of 3 cm^3. The spectrum (absorbance against the blank) during the reaction at room temperature was detected scanning in the range of 260–700 nm using an ULTROSPEC III (Pharmacia-LKB) spectrophotometer and its software. Spectrum scannings were performed in every minutes for ten minutes.

1.1.1. Enzyme. The applied laccase enzyme preparation was produced by *Lentinus edodes* in submerged cultivation. The fermentation broth was salted out and dialysed with ammonium sulphate and purified by ion exchange chromatography (DEAE Sephadex A–25) (VERECZKEY et al., 1993).

1.2. Estimation method I

To obtain appropriate estimates of turnover let us consider the basic enzyme reaction scheme:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E + P$$

where

S = substrate, P = product, E = enzyme, ES = enzyme-substrate complex, k_i = rate constant i

The usual reaction equations are obtained by the law of mass action and the material balance on the substrate in terms of concentration of the respective variables (SZIGETI et al., 1989):

$$\frac{dS}{dt} = (k_2 + k_1 S)(S^* - S - P) - k_1 E^* S \tag{1a}$$

$$\frac{dP}{dt} = k_3 (S^* - S - P) \tag{1b}$$

where $S(0) = S^*$, $P(0) = 0$, $E(0) = E^*$ are the initial conditions.

The derivative d/dS(dP/dt) can be calculated as follows. Differentiating (1b) with respect to S, we compute

$$\frac{d}{dS}\left(\frac{dP}{dt}\right) = -k_3\left(1 + \frac{dP}{dS}\right) \tag{2}$$

Substituting for dP/dS from (1)

$$\frac{dP}{dS} = \frac{k_3 (S^* - S - P)}{(k_2 + k_1 S)(S^* - S - P) - k_1 E^* S} \tag{3}$$

The limiting value as $S \to 0^+$ of (3) is known (CROOKE et al., 1986), but it can also be easily obtained by dividing both the numerator and the denominator by $(S^* - S - P)$ and using L'Hospital's rule for the last term of the denominator. The value of this limit is given implicitly as

$$z = \lim_{s \to 0^+} \frac{dP}{dS} = \frac{k_3}{k_2 + k_1 E^* [1/(1+z)]} \tag{4}$$

from which we can solve for z. The solution of this quadratic equation is:

$$m_0 = \lim_{s \to 0^+} \frac{d}{dS}\left(\frac{dP}{dt}\right) = k_3 \frac{k_1 E^* - k_2 - k_3 + \sqrt{[(k_2 - k_3 + k_1 E^*)^2 - 4k_2 k_3]}}{2k_2} \tag{5}$$

It is noteworthy that the slope determined by (5) is strongly correlated with the differential yield introduced by CROOKE and co-workers (1986) as $y = -dP/dS$. This is shown by differentiating (1b) and forming the limit value as $S \to 0^+$:

$$m_0 = k_3(y_0 - 1) \tag{6}$$

where y_0 is the limiting yield at S = 0. Therefore k_3 can be estimated from equation (6). What is specially interesting here is that this constant is obtained from stationary state data, so that the difficult to obtain early time (transient) data may not, in principle, be required to identify the unknown in the enzyme kinetic model.

1.3. Estimation method II

To obtain another estimate for k_3 let us consider equation (1b). Taking the integral of both sides then:

$$P(t) = k_3 \int_0^t (S^* - S - P)dt \tag{7}$$

It is noteworthy that if there is any possibility to use the integrated form for estimation instead of the derivative form that is offered.

If both P and S are measured in the same appropriate unit (mol or mol l^{-1}) then expression (7) is suitable to estimate k_3 because in equation (7) the integral expression (right-hand side) can be considered as independent variable and by a linear regression passing through the origin k_3 can be estimated.

Now let us suppose that neither P nor S can be measured in appropriate units, however linear functions of P and S are measurable (e.g. absorbances) i.e.:

$$S = \alpha_1\sigma + \alpha_2 \tag{8}$$

$$P = \beta_1\pi + \beta_2 \tag{9}$$

Substituting equation (8) and (9) into (1b)

$$\beta_1 \frac{d\pi}{dt} = k_3(\alpha_1\sigma^* - \alpha_1\sigma - \beta_1\pi - \beta_2) \tag{10}$$

dividing by β_1

$$\frac{d\pi}{dt} = k_3\left(\frac{\alpha_1\sigma^*}{\beta_1} - \frac{\beta_2}{\beta_1}\right) - k_3\frac{\alpha_1}{\beta_1}\sigma - k_3\pi \tag{11}$$

taking the integral of both sides

$$\pi(t) - \pi(0) = k_3\left(\frac{\alpha_1\sigma^*}{\beta_1} - \frac{\beta_2}{\beta_1}\right)t - k_3\frac{\alpha_1}{\beta_1}\int_0^t \sigma(t)dt - k_3\int_0^t \pi(t)dt \tag{12}$$

where

$$\pi(0) = -\frac{\beta_2}{\beta_1} \tag{13}$$

The last term of equation (12) contains k_3 in appropriate unit.

If S can be measured in appropriate unit or α_1 and α_2 are known as calibration coefficients i.e. S can be presented in appropriate unit then substituting (9) into (1b)

$$\beta_1 \frac{d\pi}{dt} = k_3(S^* - S - \beta_1\pi - \beta_2) \tag{14}$$

dividing by β_1

$$\frac{d\pi}{dt} = k_3\left(\frac{S^*}{\beta_1} - \frac{\beta_2}{\beta_1}\right) - k_3\frac{S}{\beta_1} - k_3\pi \tag{15}$$

and taking the integral

$$\pi(t) - \pi(0) = k_3\left(\frac{S^*}{\beta_1} - \frac{\beta_2}{\beta_1}\right)t - \frac{k_3}{\beta_1}\int_0^t S(t)dt - k_3\int_0^t \pi(t)dt \tag{16}$$

is obtained. It can be seen that k_3 can also be estimated by linear regression and furthermore β_1 and β_2 can be calculated from the regression coefficients therefore the product is "calibrated by kinetics". This is specially important in the case of fermentation products.

2. Results

The experimental design of Table 1, for the investigation of laccase activity that is for the estimation of the kinetic coefficients determining the behaviour of the reaction, was realised.

Table 1

Experimental design for the enzyme reactions. (No. 8 and 9 have the same conditions as No.10 and 11, respectively, however the later ones took longer time.)

No. of experiment	Water (μl)	Alcohol (μl)	MES (ml)	Syringaldezin (μl)	Enzyme (μl)
1	300	–	2.25	300	150
2	150	–	2.25	300	300
3	–	–	2.25	300	450
4	300	100	2.25	200	150
5	150	100	2.25	200	300
6	–	100	2.25	200	450
7	300	200	2.25	100	150
8	150	200	2.25	100	300
9	–	200	2.25	100	450
10	150	200	2.25	100	300
11	–	200	2.25	100	450

Each experiment resulted in a spectrum which is similar to Fig. 1 where the decreasing of the left-hand side peaks shows the substrate consumption and the increasing of the right-hand side peaks does the product formation. To test the above described methods both substrate and product have to be simultaneously measured. This was possible by a photometer in our case, the substrate was measured at 350 nm and the product at 550 nm. Naturally these measurements are not perfectly simultaneous and there is 13.5 s between them therefore linear interpolation was used for correction. Considering the notations of the theoretical part, the latin letters (P,S) stand for the product and substrate in moles (mol), the greek letters (π,σ) in absorbance, respectively. Each peak was measured once a minute until the substrate consumed. Figure 2 illustrates one of the experiments. It can be seen that in the cases of the slow reactions like this there is a significant difference between the initial substrate concentration (S^*) and the substrate concentration (S_P) where the product rate takes on its maximum value. (More precisely it is illustrated in absorbances i.e. σ_P, σ^*.) This is important because the Briggs-Haldane hyperbola has to pass through the maximum point of $v = dP/dt - S$ curve (CROOKE et al., 1979; CROOKE & TANNER, 1980; SZIGETI et al., 1989; SZIGETI & TANNER, 1993). The only point that $-dS/dt = dP/dt$ is at S_P.

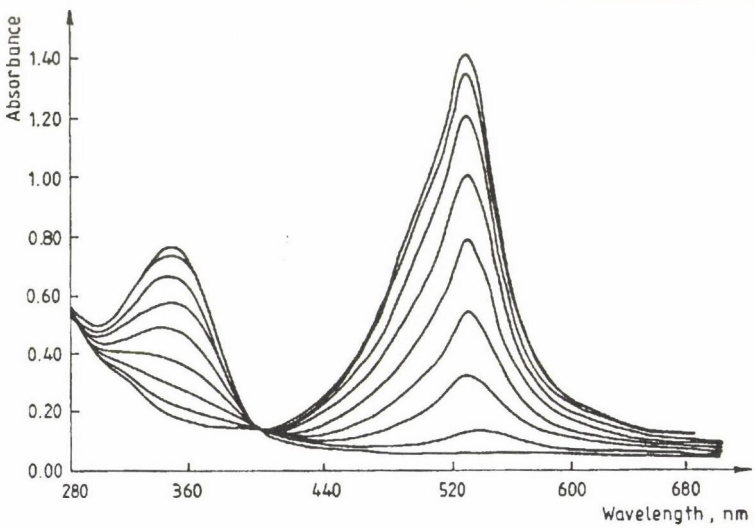

Fig. 1. Spectra of the enzyme reactions

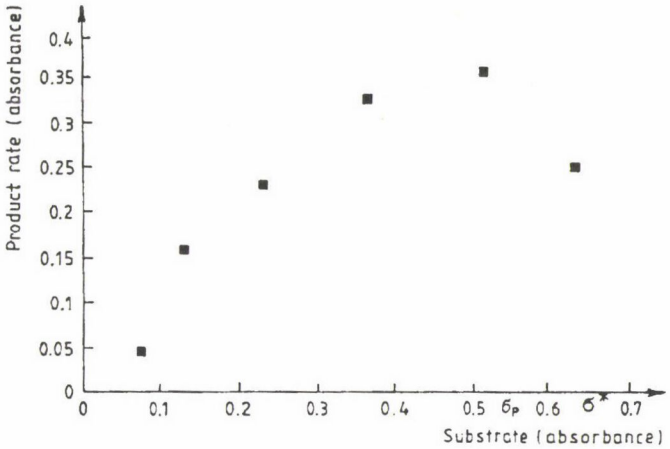

Fig. 2. An experiment in the product rate – substrate "phase plane"

Table 2 shows the experimental conditions (S*, E*) and the results (S_P, $-dS/dt$). It can be seen from the data of Table 2 that the substrate was calibrated between absorbance and μmol l^{-1}. Naturally, it is easy to calculate v_{max} and from the results for example by the Lineweaver-Burk linearization. Since k_3, β_1 and β_2, can be estimated by equation (16) therefore both E* and the calibration for P can be performed. These results are shown in Table 3. For calculating E* the integral method [Eq. (16)] was used because it resulted in less variances than the other method. (In Table 4 the k_3 estimates are shown by both dynamic estimation methods.)

Table 2

Experimental results

No. of experiment	S* diluted (μmol l^{-1})	S* calibrated (μmol l^{-1})	S_P calibrated (μmol l^{-1})	$-dS/dt$ (max) (μmol l^{-1} min^{-1})	E* (μl)	$v_0 = -dS/dt(0)$ (μmol l^{-1} min^{-1})
1	50	50.26	31.99	5.20	150	1.05
2	50	46.00	21.87	7.95	300	3.68
3	50	48.90	36.85	9.34	450	5.32
4	33.33	26.00	26.00	4.09	150	4.09
5	33.33	33.18	17.69	5.64	300	1.45
6	33.33	38.19	26.10	7.29	450	5.20
7	16.66	14.49	8.87	2.37	150	0.51
8	16.66	6.83	3.26	2.38	300	0.46
9	16.66	15.41	12.71	4.20	450	2.70
10	16.66	13.34	7.11	3.89	300	2.48
11	16.66	16.40	12.46	4.93	450	3.96

Table 3

Estimates k_3, E^* and the calibration coefficients

Enzyme µl	k_3 ($l\ min^{-1}$)	Mean k_3 ($l\ min^{-1}$)	v_{max} ($\mu mol\ l^{-1}\ min^{-1}$)	E^* ($\mu mol\ l^{-1}$)	Calibration absorbance → $mol\ l^{-1}$
150	1.999	1.213	7.968	5.569	$P = 12.21\pi$
	0.426				
300	0.726	0.975	11.362	11.650	$P = 13.94\pi - 0.64$
	1.224				
450	1.078	1.078	18.616	17.269	$P = 13.66\pi + 0.76$

3. Conclusions

Although our experience and other numerical simulations (FITZ & TANNER, 1989; SZIGETI & SEVELLA, 1994) were favourable for k_3 estimation however as this paper shows that the estimate is very sensitive to the measurement error (the method can not compensate the outlying values), therefore the variance of estimate is too large and sometimes the results do not make sense hence they are not in Table 4.

Therefore this estimation can be considered as a semiquantitative one. Maintaining the theoretical inferring analysis, we think that one of the underlying reasons is the application of the least squares method i.e. the usual regression. In the future we will apply robust regression (HUBER, 1981) for such problems.

Table 4

Estimates k_3 by both methods
(*stands for the estimates do not make "physical" sense)

No. of experiment	k_3 by the integrals Eq. (16)	k_3 by the derivatives Eq. (6)
1	0.426	0.403
2	1.224	1.287
3	*	*
4	*	*
5	*	*
6	*	0.927
7	1.999	1.361
8	0.726	0.705
9	1.077	0.758
10	*	0.549
11	*	0.703

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THE HAMLET OPTION IN FOOD MICROBIOLOGY: TO ANALYZE OR NOT TO ANALYZE FOOD SPECIMENS AS MARKETED ONCE HACCP IMPLEMENTED[a]

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Since the inception in the 1920s attempts to manage the microbiological safety and quality of foods relied almost entirely on the retrospective-analytical strategy, borrowed from food chemistry. Whereas it was effective in that instance, it failed completely to ensure microbiological food safety. This arose from the extremely limited significance of negative test results due to the sporadic and erratic character of the colonization of most processed foods and the almost perennial state of flux of the microbial community structure of the majority of foods.

Pioneers in public health in the United States, United Kingdom and France emphasized that the lack of management of microbial risks in foods, as demonstrated by the steadily increasing incidence and variety of food-transmitted infections and intoxinations, called for a different strategy, i.e. one striving for containment by intervention. A forward control approach including a processing-for-safety step applied to raw materials of animal origin, as successfully adopted in the dairy industry, was explicitly recommended.

The expert recommendations were not to a marked extent heeded until the space age. As no microbiological risks could be tolerated when feeding astronauts, Bauman introduced the now generally accepted HACCP approach: hazard analysis enabling subsequent control of critical practices. That maxim was soon extended to the entire food processing and catering industries, whereby the need for longitudinal extension – from the farm gate to the consumer's plate – was advocated.

After the HACCP strategy had culminated in numerous textbooks and course manuals, some scientists felt that the once sanctified monitoring could henceforth be abandoned. Following this swinging of the pendulum in the opposite direction would, however, introduce a novel risk. The Hamlet option: to monitor or not to monitor should definitively be answered in an affirmative way. In spite of meticulous adherence to HACCP-based good practices occasional human, instrumental or operational hiatuses can and will occur. In order to allow their rapid identification and rectification, rational monitoring of line and final product samples remains a pressing need.

As a rule, reliance can be placed upon the use of accurately quantified tests for marker organisms, whose taxonomic, physiological and ecological properties are similar to pathogens of common occurrence although they are far more abundant in the raw foods acting as the

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source of contamination of finished products. Strict adherence to previously adopted analytical precautions is also henceforth called for. This includes: (i) expertly elaborated testing of culture media before use for productivity and selectivity; (ii) carefully designed resuscitation procedures to be applied to the injured population fractions, encountered in processed foods; (iii) assessment of attainable, maintainable and adequate protection affording reference ranges ('standards'); and (iv) education and recredentialling of laboratory staff through short courses taught by professional tutors. Methods should be as simple as reliability allows, so as to enable as many samples to be examined as statistical considerations dictate.

Keywords: HACCP, microbiological food safety, monitoring for compliance, proactive safety and quality assurance, microbiological reference ranges

1. A condensed history of pursuing microbiological food safety: from backward to forward control

1.1. The pre-HACCP era

Attempts to improve the at that time prevailing microbiological safety of foods started about 1920, partly as a response to disappointing experience with foods supplied to Armed Forces during World War I (PRESCOTT, 1920). Food microbiology as a scientific discipline did not emerge before the 1930's, however (HAINES, 1933, 1939; INGRAM, 1934). Hence the first efforts in ensuring microbiological food safety were mostly made by food chemists. Consequently, the strategy to obtain microbiologically safe foods was borrowed from chemical food hygiene, on the European continent termed bromatology, from βρωμη = food. Protecting the public against chemical risks in foods – or nutritional deficiency – traditionally relied on a retrospective sentinel system. Foods as marketed were examined for toxic constituents like arsenic, mercury, lead, etc. and for stretching by the addition of adulterants of little or no nutritive value. This policy was quite successful in avoiding compounds with adverse health effects, because their absence could reliably be assessed. Inorganic and organic toxicants are rather homogeneously distributed in foods, while their concentrations remain virtually constant during storage and distribution (MOSSEL et al., 1995a).

None of these conditions apply, however, to microbiological contamination of foods or catered meals. Correctly manufactured and vended food commodities are erratically contaminated, i.e. contamination and colonization bear a stratified character; cf. Fig. 1. Moreover, the microbiological community structure in foods is in a constant state of flux. With the exception of bacterial and fungal spores in a state of dormancy, during storage and distribution of most commodities some components of the microcosm consortium are increasing in numbers of cfu, whereas other taxa decrease. This leads to perpetual population changes. Consequently, negative results of retrospective monitoring of samples lack any significance for the consignment they originate from.

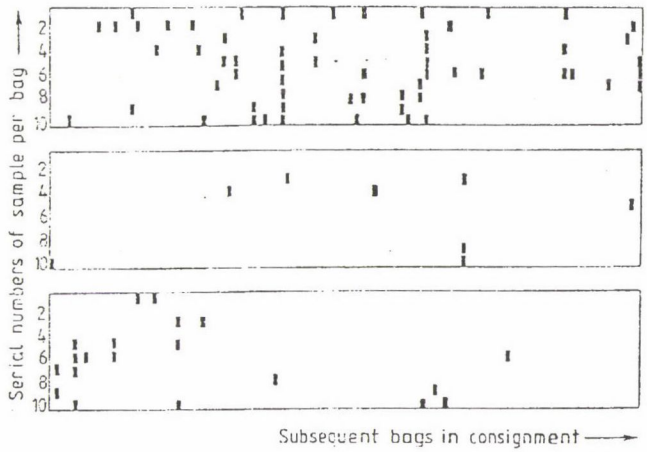

Fig. 1. Heterogeneity ('stratification') assessed during intensive sampling of three successive consignments of a dried food commodity. The black squares represent samples found to contain at least one cfu of Enterobacteriaceae; the white fields similarly represent samples wherein these bacteria were not detected

It is therefore not surprising that the backward control strategy adopted from bromatology soon proved to be totally ineffective to avert food-transmitted diseases of microbial aetiology; cf. Fig. 2. Epidemiological data from all over the world demonstrate how the retrospective paradigm borrowed from food chemistry failed spectacularly (ARCHER & YOUNG, 1988; BEAN & GRIFFIN, 1990; BEAN et al., 1990; LACEY, 1993; HOOGEBOOM-VERDEGAAL et al., 1994). This sad situation is exacerbated by the not infrequent, often most serious, sequelae of primary food-transmitted gastro-intestinal infections. These include more serious affections of the gastro-intestinal tract, but also systemic diseases, e.g. septicaemia, rheumatic symptoms and haematological disturbances such as the haemolytic-uraemic syndrome (MOSSEL et al., 1995a). Altogether this resulted in, e.g., for the USA 1994, the startling incidence data collected in Table 1. Since the 1930's leaders in Public Health issued warnings repeatedly that, in attempts to prevent food-borne infections, post-factum inspection could not be relied on; and, therefore, had to be replaced by forward intervention strategies (WILSON, 1933; DACK, 1956; BUTTIAUX et al., 1956). A quotation from WILSON (1973) illustrates this point:

> "We must keep asking ourselves whether any measure we should like to introduce would in fact appreciably diminish the incidence of ... food-borne disease. When I look back on some of the great food-poisoning outbreaks with which I have been associated I sometimes wonder whether any routine bacteriological tests ... would have prevented them: and I continue to wonder. It is ... in my opinion ... far more important to lay down

a strict code for the ... processing of food and see that it is carried out properly than to rely on bacteriological sampling of the finished product"

1.2. Bauman's paradigm shift

This message was not heeded until the Space Age. At that time foods to be taken aboard to feed astronauts had to be made fully safe: outbreaks of diarrhoea or vomiting during space travel would, obviously, have disastrous effects. This prompted Dr Howard E. Bauman, employed by a leading Food Manufacturing Industry in Minnesota, to introduce a preventive system, which he termed Hazard Analysis [allowing] Control of Critical Points [thus identified], abbreviated to HACCP.

In more detail this strategy aims at containing any hazardous processes, procedures and practices. This has to be pursued by technological procedures ensuring the following goals: (i) the elimination of pertinent pathogens; and/or (ii) curtailing the proliferation and metabolism of pathogenic and toxigenic micro-organisms. The latter goal can be achieved by adjusting intrinsic limiting factors (a_w, pH, nitrite, etc.), extrinsic inhibitory parameters (temperature, pCO_2) or antagonistic biotic activities: production of lactic acid, H_2O_2, bacteriocins and antibiotics, to inhibitory levels (MOSSEL & STRUIJK, 1992).

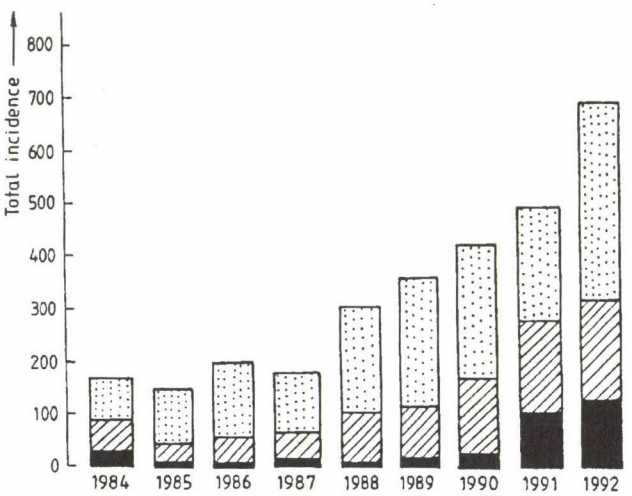

Fig. 2. The incidence of salmonellosis, campylobacteriosis and shigellosis in Leeds (UK) as a function of time. ■: Shigella; ⊡: Campylobacter; ▨: Salmonella. Source: LACEY (1993)

Table 1

The most recent North-American data on the incidence and impact of food-transmitted infections and intoxinations

The consumer as the ultimate bioassay animal for the demonstration of the lack of microbiological safety of foods. Data *per annum*

Number of outbreaks	700
Number of patients	80×10^6
Deaths	7×10^3
Cost	$\$ 20 \times 10^9$

Source: NCDC (1995), Courtesy Dr C. Hedberg, Minneapolis, Minn., USA.

Since times immemorial this strategy, *i.e.* relying on Good Manufacturing & Distribution Practices (GMDPs), has been followed in the prevention of microbial spoilage of foods. The pasteurization of milk and the curing of meat and fish products constitute examples of the efficacy of such measures of forward control (MOSSEL et al., 1995a).

1.3. The completion: Lord Plumb's maxim and its adoption by EU and FSIS (USA)

The HACCP maxim was originally centered around safeguarding the industrial production of food (BAUMAN, 1974). It was, somewhat later, particularly under the influence of Lord Hugo Plumb of Coleshill (MOSSEL et al., 1995a) extended to longitudinally integrated safety assurance (LISA) from the farm gate to the consumer's plate (ESPY, 1994), sensu DACK (1956).

In 1993 the European Union added the dimension of autonomous total quality assurance (ATQA), summarized in Table 2. This legislation, which became effective late December 1995 (GRIJSPAARDT, 1995), entrusts the prime responsibility for compliance with HACCP-based GMDPs to the Food Manufacturing and Catering businesses themselves (MOSSEL et al., 1995b). Most recently USDA's Food Safety and Inspection Service has proposed a similar paradigm shift. It will require, e.g., for the meat industry (i) to introduce a system of consistent plant hygiene, a decontamination step and additional preventive controls, including rapid chilling and low temperature storage regimes; and (ii) to assign a scientist of demonstrable ability responsible for specimen collection, their bacteriological examination by approved methods, and recording of the results (HALL, 1995).

Table 2

The autonomous total quality assurance (ATQA)-trilogy aiming at ensuring safety, quality and acceptability of foods and catered meals

Stage 1: Strategy

Design of modes of elimination of all identified critical sites and practices relying on holistic quantitative risk analysis – HACCP.

Stage 2: Implementation

Implementation of the required intervention steps all along the production, distribution and culinary preparation line – LISA.
Given the actual severe microbiological contamination of the farm, abattoir and estuary environments, these include almost invariably a processing-for-safety step.

Introduction of the products to the public, supported by professional safety communication, emphasizing their safety and sensory attributes ('quality') and, where applicable nutritive value – SQA.

Education and motivation: Interdependent encouragement.

Stage 3: Codification & Validation

Meticulous codification of procedures to be followed throughout: publication of Good Manufacturing and Distribution Practices – GMDPs.

Adequate monitoring, to validate functioning of ATQA without hiatuses, and record keeping of results. Use of Standard Operating Procedures with results gauged against Reference Ranges for.
AQL = acceptable quality level
TSL = tolerable safety limit

Sources
European Union Directive 93/43, 14th June 1993. Offic. J. Eur. Comm. 19.7.1993. No. L175. 1–6. Cf. Jacob, M. 1994. Internat. Food Safety News 3. nr. 7. 80

2. The pendulum swings too far

2.1. Contra versus pro monitoring

Whereas previously reliance was entirely placed on monitoring of food samples, upon the enthusiastic acceptance of the LISA strategy many food science practitioners surmised that henceforth laboratory examinations would be a thing of the past. This was most dramatically displayed by the strong movement against the use of 'standards' of microbiological nature. It is quite obvious that microbiological inspection of food and food environment specimens without having reference data available allowing data obtained to be gauged against, is a fruitless procedure. Hence, denying microbiological limit values to be elaborated and used constitutes rejection of examination *per se*.

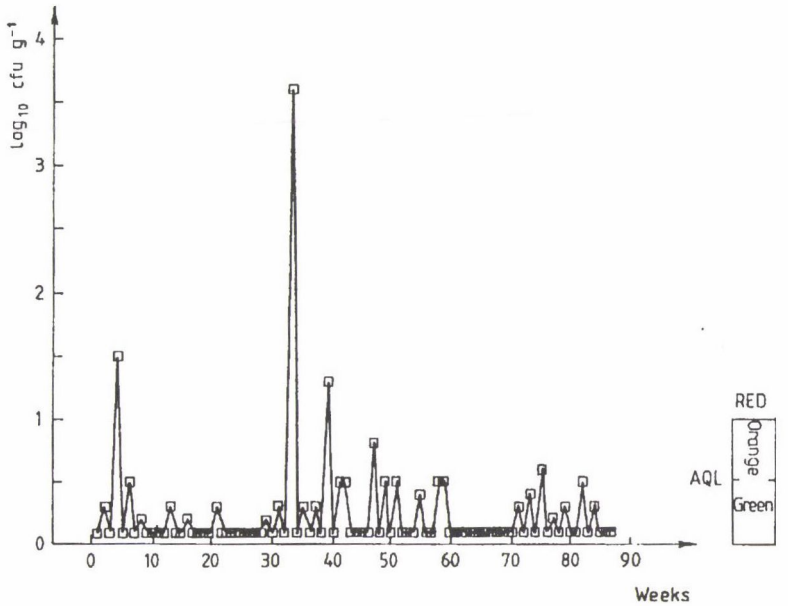

Fig. 3. Trend chart documenting the course of the detection of a given marker organism in a food processed-for-safety. Generally "negative" results of routine testing of final product samples will be obtained in a well-run plant, unless incidental hiatuses occur

Beyond a shine of doubt, spot checks of microbiological-analytical nature will remain a pressing need. Despite strict adherance to codified LISA/ATQA-practices, occasional hiatuses will occur, though systematic failures will of course be avoided. These incidental imperfections, illustrated by Fig. 3, are due to minor instrumental failures, or unfortunate human inaccuracies. They can be rapidly identified – and more importantly no less swiftly remediated – by expert monitoring procedures. The majority of these measure process and strategy parameters, such as time/temperature integrals, a_w, pH, etc. However, a fair amount of, if at all possible real-time, or at any rate rapid, direct microbiological testing remains mandatory.

2.2 Why and how to overcome the aversion?

It is legitimate to wonder, why this so rational maxim needs defending it against opinions to the contrary.

The reason for this is, that in too many instances monitoring has been suggested for purposes where it would not serve. Moreover, occasionally methods have been recommended that are inadequate, inaccurate, outdated, or all three at the same time. The latter is not too surprising. Chemical examination of foods dates back to over 120 years and was in the hands of one discipline, whereas scientifically founded microbiological analysis of foods is conducted since about 1960 only and

emerged from no less than six different disciplines: medicine, microbiology, pharmacy, chemistry, veterinary medicine and food science (MOSSEL et al., 1995a).

Indeed, there is a need for every single test to assess, before it will be adopted or even suggested for use, whether it is warranted, accurate, efficacious, cost-effective and provides results within a useful time-span. Even so, the numbers of criteria employed to validate adherence to GMDPs should always be restricted to the minimum that is epidemiologically justified and ecologically valid. Nothing has been more disastrous to the acceptability of microbiological monitoring than standard freaking! Often, long 'shopping lists' of impressive criteria were designed by scientists who had, for quite a period of time, lost contact with the practice of Food Science. For instance, for some foods of marine or estuarine origin, tests for *Shigella* spp. were prescribed notwithstanding that from most foods in commerce, wherein they may occur, these bacteria cannot routinely be isolated as a result of their predominantly negative attributes (JUNE et al. 1993; FLODERUS et al., 1995; KAPPERUD et al., 1995).

A final convincing argument for their adoption is that laboratory monitoring, linked to general compliance with GMDPs, is not a laborious exercise at all. In a well run operation, results of appropriately designed tests will be "negative", as also documented in Fig. 3. Hence little subculturing, galleries, serology, etc. will be required.

3. Analytical facets to be taken into account

3.1. Selection of criteria

3.1.1. Target-organisms. In principle very few searches for pathogenic organisms will be necessary for verifying compliance with HACCP-derived GMDP-guidelines. Suitably chosen marker organisms will, as a rule, suffice for that purpose. Marker organisms may serve two purposes.

First of all they allow assessing whether processing-for-safety has been carried out properly. The coli-acrogenes group of Enterobacteriaceae has been applied for that purpose to milk and ice cream. Markers can also render services to estimate the probability of the presence of pathogens, when selected meticulously, taking into account ecological fundamentals. Markers intended to be used for this purpose should bear taxonomic and physiological resemblance to the target pathogen, while being much more abundant in the raw material from which that pathogen is to be eliminated. As illustrated by Fig. 4, the thermotrophic segment of the Enterobacteriaceae constitutes a reliable marker for *Salmonella* spp. in many processed foods and animal feeds (MOSSEL et al., 1986; VAN DE MOOSDIJK et al., 1989, VELDMAN et al., 1995).

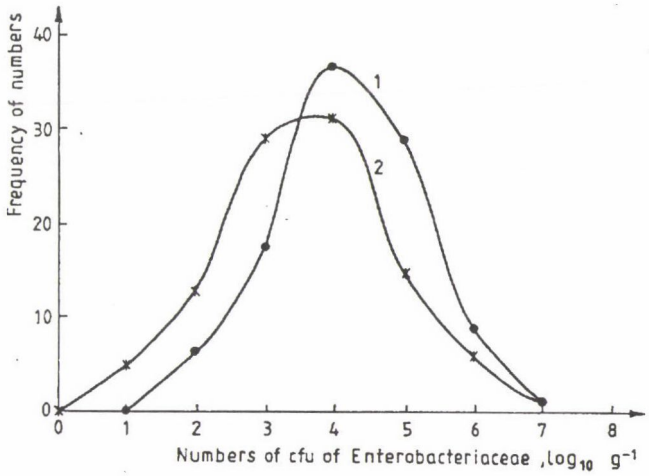

Fig. 4. Distribution plot of the numbers of Enterobacteriaceae for *Salmonella*-positive (1) and *Salmonella*-negative (2) samples of mixed animal feeds. Source: VELDMAN et al. (1995)

Clearly, testing for pathogens cannot be missed in epidemiological investigations and in the final, confirmative stage of predictive modelling (MCMEEKIN et al., 1993; BREMER & OSBORN, 1995).

3.1.2. Quantification. Limit values for micro-organisms include acceptable quality levels (AQLs) for markers and agents of spoilage and, to a lesser extent, tolerable boundary tests for pathogens. Acceptance ranges should not be invented behind a desk, nor arrived at by negotiation around a conference table. They should rather be derived empirically – as in internal medicine – from surveys establishing 'normality'. In terms of microbiological food safety this denotes the microbiological condition resulting from adequate risk management, which is attainable and maintainable by contemporary technological practice (MOSSEL & VAN NETTEN, 1991).

In all instances allowance should be made for the stratified distribution of target organisms (*vide supra*) and for the intrinsic variability of boundary (presence-or-absence) tests, often used in the assessment of compliance; *vide infra*. Hence, certain tolerances are mandatory when specifying AQLs. This has been acknowledged by the introduction of the three-class-acceptance/rejection maxim by BRAY and co-workers (1973), documented in Fig. 5.

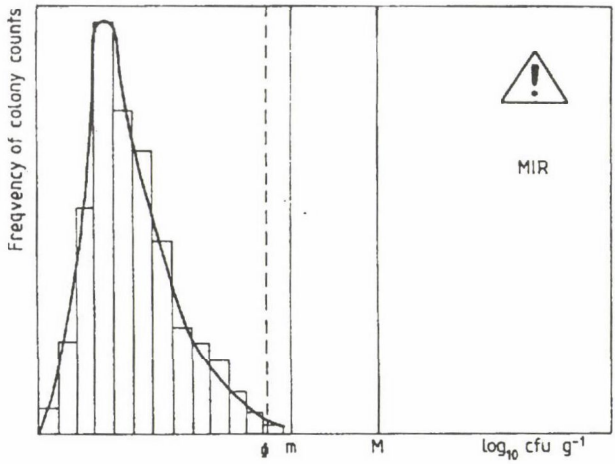

Fig. 5. Frequency distribution of pertinent colony counts in foods produced under rigorous adherence to Good Manufacturing and Distribution Practices (GMDPs), allowing categorization in three classes: <m = Reference value proper; m→M = zone of tolerance; > M = not to be expected in the absence of non-structural hiatuses. MIR = Minimal Infectious Range of pathogen of concern in the food under review.

3.2 Practical details

3.2.1. Ensuring representative sampling and specimen homeostasis. Sampling consignments of foods has attracted great interest and prompted many research efforts, as its adequacy markedly affects the accuracy of data obtained. It is now generally realized that a major discrepancy between consignment and sample may render monitoring totally futile (ICMSF, 1986).

It may be wise to remember also the warning, issued over three decades ago, that, in addition, measures have to be taken to ensure that the microbial consortium, present in the food at the moment of sampling will not significantly change during transportation, storage or sample preparation, as a result of exposure to temperatures or gaseous atmospheres that give rise to marked flora shifts (MACKENZIE, 1960, TE GIFFEL et al., 1995). The design of such measures calls for thorough ecological and physiological knowledge of any particular target organism or consortium.

A warning, never to ignore measures aiming to avoid cross-contamination of specimens may neither be superfluous (JOCE et al., 1995).

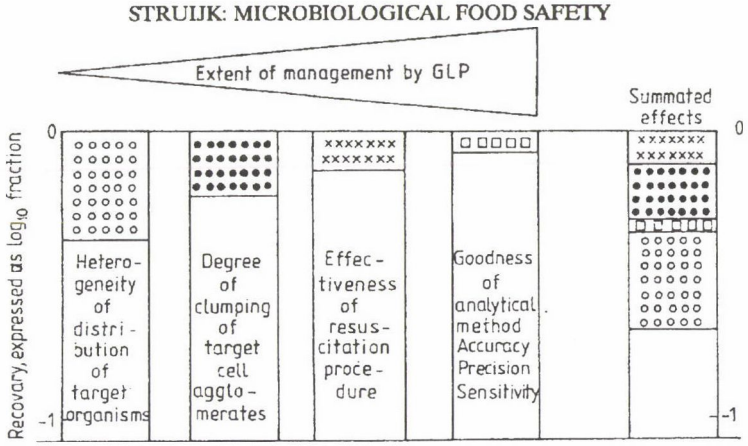

Fig. 6. Phantom histogramme illustrating the impact of control, by Good Laboratory Practices (GLPs), i.e. strict adherence to accurately codified standard operating procedures, on the reliability of the recovery of target organisms from a food matrix

3.2.2. Accuracy and precision. As emphasized before, a few analytical parameters may substantially reduce the consistency of data obtained in the microbiological monitoring of the food environment and commodities as marketed or ingested. These include (i) hard to overcome adhesion of target organisms to food contact surfaces and food matrices; (ii) clumping of cell agglomerates; (iii) their stratified distribution; (iv) inhibition of undisturbed development of target organisms by other microbes occurring in the food, but devoid of significance; cf. Fig. 6; and (v) deficiencies in aseptic precautions during manipulation of samples (JOCE et al., 1995). Even if the adverse effects of these phenomena are limited to the highest possible extent, variation coefficients of colony counts may well amount to 10%, while the confidence interval of P-A-tests may span an entire log. cycle (MOSSEL et al, 1995a).

In a few instances one or more of these factors so adversely affect repeatability and reproducibility of a given test that its use in practice would be unwise since it would lead to perpetual conflicts within or between laboratories. Available methods should then be improved, or a better, i.e. more consistently determinable, criterium be selected.

3.2.3. Performance testing of media. One of the most serious, though fully containable, shortcomings of a method would be ignoring the need for quality monitoring of any liquid or solid culture medium used (WEENK et al., 1992; ORR et al., 1995). This was hardly practised, however, before 1970 (MOSSEL, 1971). Fortunately, as demonstrated by the catalogues of, and the labels used by, medium manufacturers, monitoring culture media for productivity, selectivity and adequate visualization, where media rely on diagnostic traits, has become standard practice since about 1980 (CORRY, 1982; CORRY et al., 1986).

3.2.4. Including sublethally stressed cells in detection methods. Early this century it was demonstrated that microbial cells that, within a given population, had survived stress of intrinsic or extrinsic nature, as a rule were sublethally damaged. This would be manifested by markedly increased lag times and failure to thrive in selective media, that allow unhindered growth and metabolism of fully vital populations of the same taxon (MOSSEL & VAN NETTEN, 1984; RAY, 1989; ALEXANDROU et al., 1995; HILLS & MACKEY, 1995). Injured cells retain their pathogenic attributes, which can be demonstrated by inoculation experiments with animals hospitable and sensitive for a given genus or taxon. They can be restored to full viability by so-called resuscitation treatments, as documented in Fig. 7, taking into account the cytological and/or physiological character of the damage incurred (KNABEL & THIELEN, 1995). In some instances the procedure to be followed may still require some further investigation (NILSSON et al., 1991; RAVEL et al., 1995).

Failure to apply deliberate resuscitation treatments to stressed populations will of course lead to severe underestimation of survivors and hence to entirely false estimates of risks of health and/or spoilage. It will also very negatively affect the consistency of analytical results, because some – very partial and erratic – fortuitous recovery will occur as a result of the preparation of food macerates and dilutions, as illustrated by the lower part of Fig. 7.

3.2.5. Pressing need for staff retraining and recredentialling. Food microbiology is, as emphasized before, a rather young and hence rapidly developing branch of biological sciences. Moreover results of progress made are reported in some 20 different scientific and technical journals. It is hence virtually impossible to remain informed of all new developments by literature study and attending an occasional scientific meeting.

'Staling' of intellectual level can, and has to be, controlled by attending, periodically, in-residence refresher courses. To allow improving awareness of newly acquired knowledge, such courses should be taught, in an educational environment, by a faculty consisting of experienced academic specialists. It has to be recruited partly on the basis of their (i) demonstrated affinity to teaching; (ii) relying on literature-alertness. Faculty should be encouraged to assess the efficiency of their theoretical and practical classes by a modest amount of examination of attendees. The latter may be further motivated to spending the tuition fee invested well by being offered an opportunity to receive a certificate, if the course has been completed satisfactorily.

A most pressing element of such courses is constituted by an expert, unbiased evaluation of the practicability of novel instrumental detection methods relying on molecular-microbiological attributes. Specialist faculty members should ensure a fully quantitative, 'hands-on' appreciation of 'rapid', including allegedly 'real-time', methodology (MOSSEL et al., 1994). Course graduates can thus profitably advise their

own laboratory directors on procuring – mostly expensive – instrumental tools and manufacturers of the latter on the need for particular improvements.

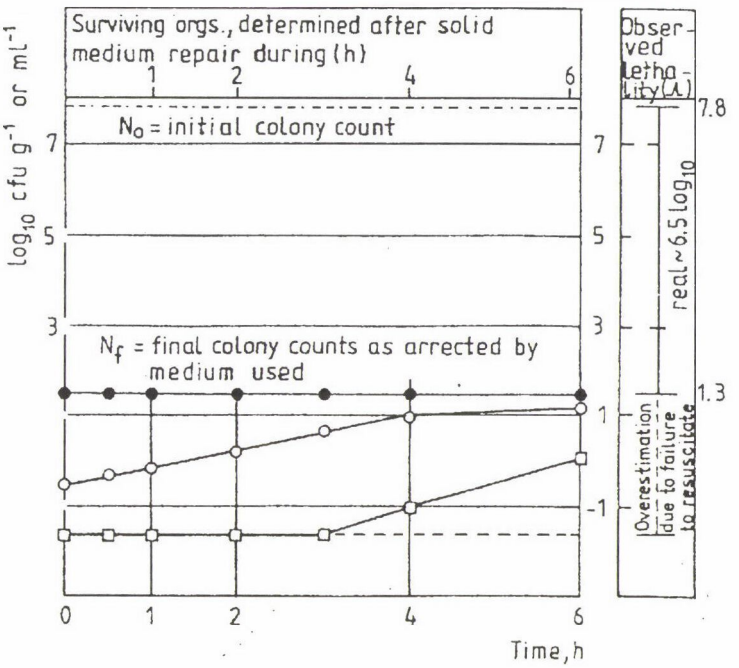

Fig. 7. Destruction-repair (DR) functions. Legend: ●: colony count obtained on a non-selective, optimal recovery medium, with as well as without resuscitation; o: *ditto* obtained with an optimal selective medium, obviously markedly affected by resuscitation treatment; □: *ditto*, obtained with suboptimal selective medium. Note: In some instances no better selective enumeration medium than the suboptimal one referred to in the figure is yet available. A procedure to assess the true rate of survival has then to be elaborated

4. Retrospect and prospects

The facts presented above allow a rationale in responding to the Hamlet option: after adoption of HACCP/LISA/GMDPs to analyze or not? The educated reply is: definitively yes.

The long overdue protection of the mostly ignorant consumer collective against fully preventable food infections and intoxinations in this sense certainly bears no revolutionary character either. It is strictly modelled after the most successful contemporary strategy adopted in internal medicine to heal the equally ignorant individual.

Similarly, in the practice of food protection the strategy of internal medicine could profitably be adopted. Hazards are first of all to be accurately identified and

their control subsequently ensured. The success or failure of remediation should be assessed by expertly elaborated laboratory procedures; in the latter case rectification follows without delay. In microbiological food monitoring, as in clinical medicine, we should strive for simple, reliable and rapid tests, in attempts to control cost and speed-up intervention when required. The procedure to elaborate 'normality', adopted in internal medicine, has already been copied in the contemporary design of rational Reference Ranges (MOSSEL & VAN NETTEN, 1991).

Four observations and arguments incite to predict, that an intelligent response to the Hamlet option will constitute the paradigm that will be accepted and implemented:

- with respect to the younger generation of food microbiologists, experience with graduate students of different types of initial academic education in various European countries, indicates that they feel quite comfortable with the ATQA-concept and the ensuing role of monitoring;
- as for the senior professional group: if they enthusiastically adopt novelties in communication technology why not similarly go for this new approach to monitoring, complementary to quality assurance;
- thirdly, there is the mundane point: ATQA saves lots of money for any corporation, because it substantially reduces the incidence of substandard consignments leaving the factory and the ensuing recalls;
- finally, as for the smaller companies, the strategy advocated in this presentation enables rational monitoring at all – which will anyway soon be mandatory world-wide.

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CONTINUOUS ACETIC ACID POLYACRYLAMIDE GEL ELECTROPHORESIS AS A TEST FOR DETECTION AND DETERMINATION OF COMMON WHEAT IN DURUM WHEAT

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A method was proposed to detect and determine the content of common wheat in durum wheat. It is based upon the acetic acid polyacrylamid gel electrophoresis (PAGE) of gliadins, followed by the determination of the specific gliadin bands which are only present in common wheat. Gliadins were extracted with 70% ethanol from 27 durum and 33 common wheat cultivars collected from several sources as well as 96 blend prepared by mixing 4 durum with 4 common wheat at 6 ratios. Durum wheat electrophoretic patterns lack certain slow moving gliadin bands present in common wheat, but these bands can be detected in the samples of durum wheat mixed with common wheat. The densities of these bands are proportional to the percentage of common wheat in the samples. Coomassie Blue of these bands was extracted overnight with 25% pyridine in water and the transmittance of the blue color was measured at 605 nm. There was a highly significant negative correlation between the transmittance values and the percentages of common wheat in durum wheat ($r = -0.93^{***}$, $n = 96$). By this method, durum wheat or its semolina samples mixed with 10% common wheat or its flour could be visually detected from the electrophoregrams. Also one can compute the percentage of common wheat in a mix from the calibration curve.

Keywords: durum wheat, gliadin proteins, electrophoresis

The quality of pasta products processed from 100% durum wheat (*Triticum durum*) semolina is most often superior to pasta in which common wheat (*Triticum vulgare*) semolina is incorporated. This is because durum semolina have low amounts of certain proteins (gluten) that make stiff dough which are better suited to the modern pasta-manufacturing and pasta with the ultimate good cooking quality. Durum semolina is also rich in yellow pigments, which impart an appealing yellow color. This explains the brisk demand for high-quality raw materials and why durum wheat semolina is more expensive than other flours in the world market. Consequently, there is economic incentive to adulterate durum semolina with cheaper flours. For these reasons, in some countries like France, Italy and U.S.A., there are laws prescribing that pasta must be made from durum wheat only, and the

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incorporation of common wheat in pasta products is considered adulteration. In Egypt, high-quality industry depends mainly on the imported durum semolina, which may be adulterated with common wheat flour. Therefore, this work was done to detect the adulteration of durum semolina.

So far, many methods have been proposed to detect the presence of *Triticum vulgare* in durum wheat, and its products. Methods of differentiation based on lipid analysis (sitosterol palmitate) were the earliest to be used. Most of these methods are derived from MATVEEF's method (1952), but as stressed by FRUCHARD and co--workers (1967), these methods lack specificity for many common wheat which contain approximately as much sitosterol palmitate as the durum wheat. In U.S.A., to assure that the pasta is 100% durum, the government uses a color test based on the presence of the yellow carotenoid pigments in the pasta (A.A.C.C., 1983, GENERAL SERVICES ADMINISTRATION, 1975, UNITED STATES DEPARTMENT OF AGRICULTURE, 1982). However, this test is flawed, since the standards are too low and pasta with high levels (up to nearly 100%) of nondurum wheat has sufficient pigmentation to pass the test, also, wheat cultivars vary in pigment content (ALAN et al., 1985). Several authors have proposed methods to determine the amount of common wheat in pasta products by estimating specific soluble proteins (PENCE et al., 1954; FEILLET & BOURDET, 1964, 1967). Moreover, FEILLET and KOBREHEL (1974) reported a method based on measuring specific water-soluble polyphenoloxidase activity after slab polyacrylamide gel electrophoresis in basic buffer. By using an aluminium lactate PAGE buffered system, LOOKHART and co--workers (1982) and JONES and co-workers (1982) tested the electrophoretic gliadin patterns of 88 common and durum wheat cultivars grown in U.S.A. They found that all nondurum cultivars tested had at least one gliadin band, which migrates slower than does the slowest durum gliadin band. Identical results have been obtained from ground pasta and wheat flour (ALAN et al., 1985).

In this paper a method is proposed for detecting durum wheat adulteration and measuring the concentration of specific gliadin bands as related to the common wheat content in durum wheat samples after slab PAGE in acetic acid buffer (CLEMENTS, 1988) as modified by TAHA (1992).

1. Materials and methods

1.1. Materials

Out of the 60 grain samples employed in this study durum wheat cultivars and 33 were common wheat cultivars. Sixteen cultivars of durums from several world sources have been supplied by ICARDA, and adapted under the Egyptian conditions by the Agronomy Dept., Fac. of Agric., Zagazig Univ., Egypt. Five Hungarian durum

and 23 common cultivars were provided by Wheat Division, Cereal Research Institute, Szeged, Hungary. The other 6 durum and 10 common wheat cultivars were Egyptians, obtained from the seed Division, Ministry of Agriculture Cairo, Egypt. All chemicals used were of analytical grade. Distilled deionized water was used to prepare gliadin extracts and electrophoresis solutions.

1.2. Milling and blends preparation

Grain samples were finely milled on a Sienceware, Bel-Art Products micro mill. Ninety six blends of 4 durum wheat and 4 common wheat with 6 ratios: 100:0, 90:10, 80:20, 60:40, 40:60 and 0:100 (w/w) were prepared.

1.3. PAGE of gliadin

The methods of gliadin extraction, gel preparation and electrophoresis were adopted from CLEMENTS (1988) as modified by TAHA (1992).

1.3.1. Reagents. The buffer and the solutions for gliadin extraction, gel preparation, protein fixation, gel staining and destaining were prepared as shown in Table 1.

1.3.2. Gliadin extraction. Gliadin proteins were extracted from 0.2 g of whole meal with 0.4 ml of 70% aqueous ethanol in 10 ml centrifuge tube. After brief agitation on a vortex mixer, the suspensions were left to stand at room temperature for 1 h, 0.4 ml of gliadin extract dilution solution (Table 1, No. 4) was added and agitated again, then centrifuged at 4000 rpm for 15 min. The red colored supernatant was transferred into a 2 ml sealed vial and stored at −20 °C.

1.3.3. Electrophoresis apparatus. The electrophoresis apparatus was a DESAPHOR VA vertical slab gel electrophoresis cell consisting of two buffer reservoirs of 1.5 l (upper) and 5 l (lower), using 25 cm wide × 15 cm long glass plates separated by 2 or 1.5-mm spacers and 24 or 29 well combs, with a circulating bath (DESAGA FRIGOSTAT) and power supply (DESATRONIG 500/500) from DESAGA, Heidelberg, Germany.

1.3.4. Gel preparation. Solutions for gels containing 12% total acrylamide with 3% cross linkages (12% T, 3% C) were prepared individually as shown in Table 1, and deaerated under vacuum using an oil pump and cooled until partially frozen. The solution was gently warmed with tap water or with hands, if necessary, until the last trace of ice disappeared, then, hydrogen peroxide was added with gentle swirling. The solution was poured rapidly into the precooled gel former and the comb was inserted immediately. The polymerization occurs within one minute after addition of hydrogen peroxide.

Table 1

Recipes for tank buffers and solutions[a]

No	Component	Quantity
1	Upper reservoir buffer	
	Acetic acid (glac.)	2.2 ml
	Water	1.4 l
2	Lower reservoir buffer	
	Acetic acid (glac.)	13 ml
	Water	5 l
3	70% ethanol in water	
4	Gliadin extract dilution solution	100 ml
	Glycerol	50 ml
	Pyronine - G	0.1 g
	Upper reservoir buffer (sol. 1)	50 ml
5	Gel solution	070.00 ml
	Acrylamide/bisacrylamide, 32% T, 3% C[b]	026.25 ml
	(31 g acrylamide + 1 g bisacrylamide + water to 100 ml)	070.0 mg
	Ascorbic acid	000.55 ml
	Acetic acid (glac.)	008.75 ml
	Ferrous sulfate, 0.032% (32 mg $FeSO_4$, $7H_2O$ + Water to 100 ml)	032.00 ml
	Water	175.00 ml
	Hydrogen peroxide, 0.6% (500 ml 30% H_2O_2 + 25 ml water)	175.00 µl
6	12% TCA in water	
7	0.5% Coomassie Blue R-250 in water	
8	Water/methanol/acetic acid, 40/50/10 (v/v/v)	
9	Water/methanol/acetic acid, 88/5/7 (v/v/v)	
10	25% pyridine in water	

[a] According to CLEMENTS (1988) as modified by TAHA (1992); [b]T: Total acrylamide concentration, C: Bisacrylamide concentration as percent of total acrylamide

1.3.5. Electrophoresis. Each well contained a layer of 10 or 15 µl of extract. The electrophoresis was run toward the negative pole at 10 mA per gel up to 30 min, followed by 6.5 h at 20 mA per gel (constant current) with temperature maintained at 20 °C. The pyronine front and the fast moving proteins pass completely off the gel during the run.

1.3.6. Staining and photography. The gel were carefully removed from the glass plates and fixed in 300 ml of 12% trichloroacetic acid (TCA) for 30 min. Then, 5 ml of aqueous 0.5% Coomassie Blue R-250 was added. Bands appeared within 1 h, but the gels were allowed to stain overnight with continuous gentle agitation. Additional stain (5 ml) was added daily over 1–2 days. The staining solution was then poured off and the sides of the tray and upper surface of the gel were washed quickly with a jet of acetone from a wash bottle. The gels were destained with two short (2–3 min) rinses with water:methanol:acetic acid, 40:50:10 (v/v/v), followed by agitation in water:methanol:acetic acid, 88:5:7 (v/v/v), for 1–4 h and photographed immediately (LAEMMLI, 1970).

1.4. Determination of common wheat content

The stained slow moving gliadin bands which are specific for common wheat (Figs. 1–3) were cut out, transferred into 10 ml tube, 2.5 ml of pyridine 25% in water was added and extracted overnight on a shaker. Then the transmittance of Coomassie Blue colored solutions was measured spectrophotometrically at 605 nm wavelength. The relationship between transmittance values and the corresponding percentages of common wheat in durum wheat samples was calculated and a calibration curve was obtained using a drawing computer program.

2. Results an discussion

Figures 1 and 2 showed PAGE patterns of gliadins extracted from 25 durum wheat cultivars and 33 common wheat cultivars side by side on 3 gels. Although wheat cultivars were obtained from several sources around the world and grown under different climatic conditions, it is obvious that electrophoregrams of durum wheat are clearly different from those of common wheat. None of the tested durum cultivars has slow moving gliadin bands (less than 0.20 relative mobility), whereas all common wheat cultivars has at least two slow moving bands (0.18 and 0.20 relative mobilities, pointed with arrowheads). This confirms that gliadin components of wheat are essentially genotypic characteristics and qualitatively unaffected by environmental factors or growing conditions (FEILLET & BOURDET, 1967; WRIGLEY & SHEPHERD, 1973).

The above result is in good agreement with that of JONES and co-workers (1982) obtained from 88 most commonly grown U.S.A. wheat cultivars, including durums, and that of ALAN and co-workers (1985) obtained with ground pasta and wheat flours.

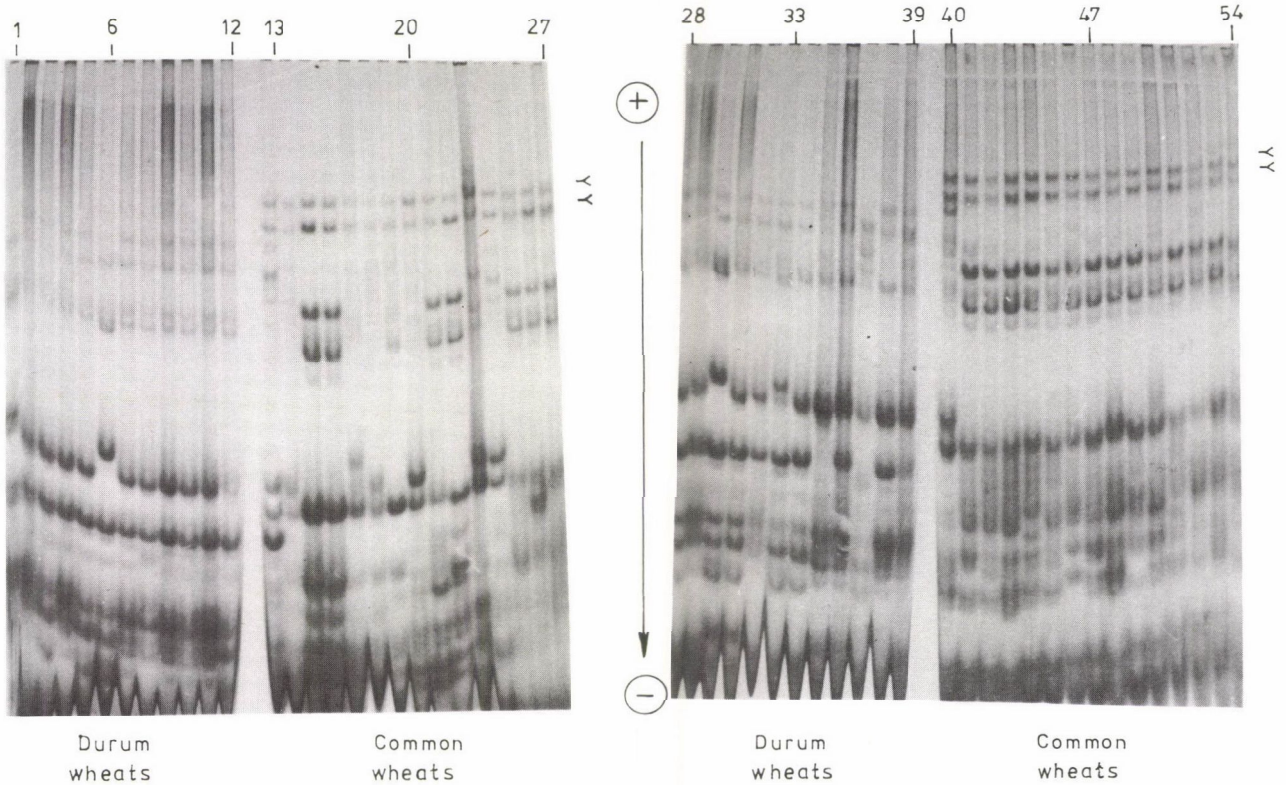

Fig. 1. PAGE patterns of gliadins extracted from 24 durum wheat cultivars abreast with 30 common wheat cultivars using a continuous acetic acid system on 12% acrylamide and 2 mm thick gel, 15 μl sample loading. Electrophoresis was run at 10 mA for 30 min followed by 7 h at 20 mA per gel and 20 °C temperature maintained. Durum cultivars: (1) Cham 1, (2) Om Rabi 5, (5) Cedifla, (7) Ruff, (10) Aw1/Mrb 15, (11) Aw12/Bit, (12) Ru/Mrb 18 and (29) Ru/Mrb 15 were from Syria; (3) Tensift 1, (6) Chahda 88, (8) Snip/F 9/Ato, (28) Cham 3, (30) Daki and (31) Ofm/Somo were from Mexico; (4) Om Rabi 9 and (9) Om Rabi 3 were from Libya; (32) Suhag 1, (33) Suhag 2 and (34) Bani Sweaf 1 are Egyptians and (35) Tiszadur, (36) Minaret, (37) Pannondur, (38) D 8-91 and (39) D 11-91 are Hungarians. Common wheat cultivars: (13) G 155, (14) G 160, (15) G 162, (16) G 164, (17) Sakha 8, (18) Sakha 61 and (19) Sakha 69 are Egyptians and (20) Góbé, (21) Mv 109-88, (22) GK-Bence, (23) GK-Örzse, (24) GK-Szke, (25) Kalangya, (26) Mv 119-88, (27) Hel-CCM×Hel-Btyl 11 m, (40) Ko, (41) Mv 14, (42) Mv 213-88, (43) Mv 10-88, (44) Zo, (45) GK 1035, (46) 160 F, (47) Mv 32-87, (48) 4 D An 6, (49) Sp-79, (50) Mv 15, (51) Csrös, (52) Maros 60-88, (53) Posavka-Li and (54) Bu-So 241, are Hungarians

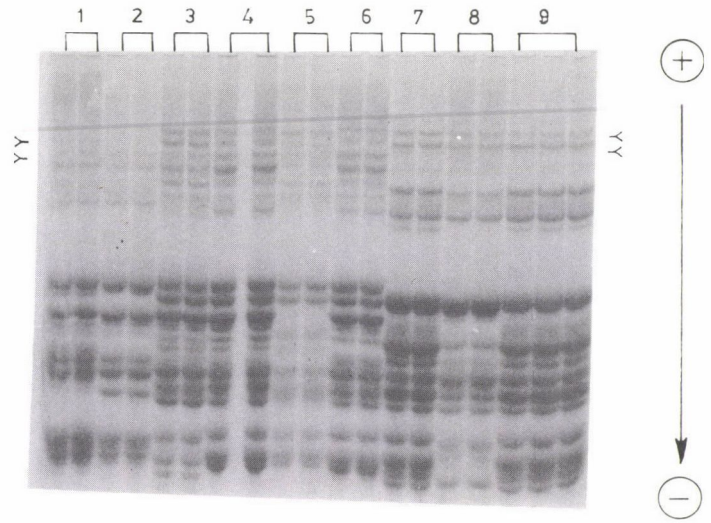

Fig. 2. PAGE patterns of gliadins extracted from durum and common wheat cultivars grown in Egypt abreast on 12% acrylamide and 1.5 mm thick gel, 10 µl sample loading and electrophoresis at 10 mA for 30 min followed by 6.5 h at 20 mA per gel and 20 °C temperature maintained. Cultivars: (1) Suhag 2, (2) Suhag 3 (durum); (3) G 155; (4) G 157; (5) G 160; (6) G 162; (7) G 163; (8) G 164 and (9) G 165 (common wheat)

According to KOBREHEL and GAUTIER (1973), the slow moving bands – which characterize the common wheat – seems to be due to genom D present in common wheat and absent in durum wheat. Genom D is known to control synthesis of some gliadins (KONZAK, 1977).

Figure 3 showed PAGE patterns of gliadins extracted from 96 blends prepared with mixing 4 durum and 4 common wheat meals at 6 ratios on 4 gels. Nondurum gliadin bands are clearly present in the blend extracts that contains only 20% common wheat, and faint nondurum bands can also be seen in 10% common wheat extracts. Test sensitivity might be increased by increasing amount of sample loaded to 20 µl instead of 10 µl. Pure durum and common wheat extracts are shown for comparison. Identical results were obtained by blending of each one of the 4 durum with each one of the 4 common wheat cultivars tested.

Figure 4 showed the relation between the transmittance values at 605 nm of the blue colored solutions obtained by overnight extraction with 25% pyridine of Coomassie Blue stained slow moving bands specific for common wheat and the corresponding percentages of common wheat in durum wheat samples. A highly significant negative correlation is clearly obvious ($r = -0.93^{***}$, $n = 96$). The percentages of contamination in durum wheat or semolina samples with unknown amounts of common wheat could be easily estimated from the calibration curve (Fig. 4).

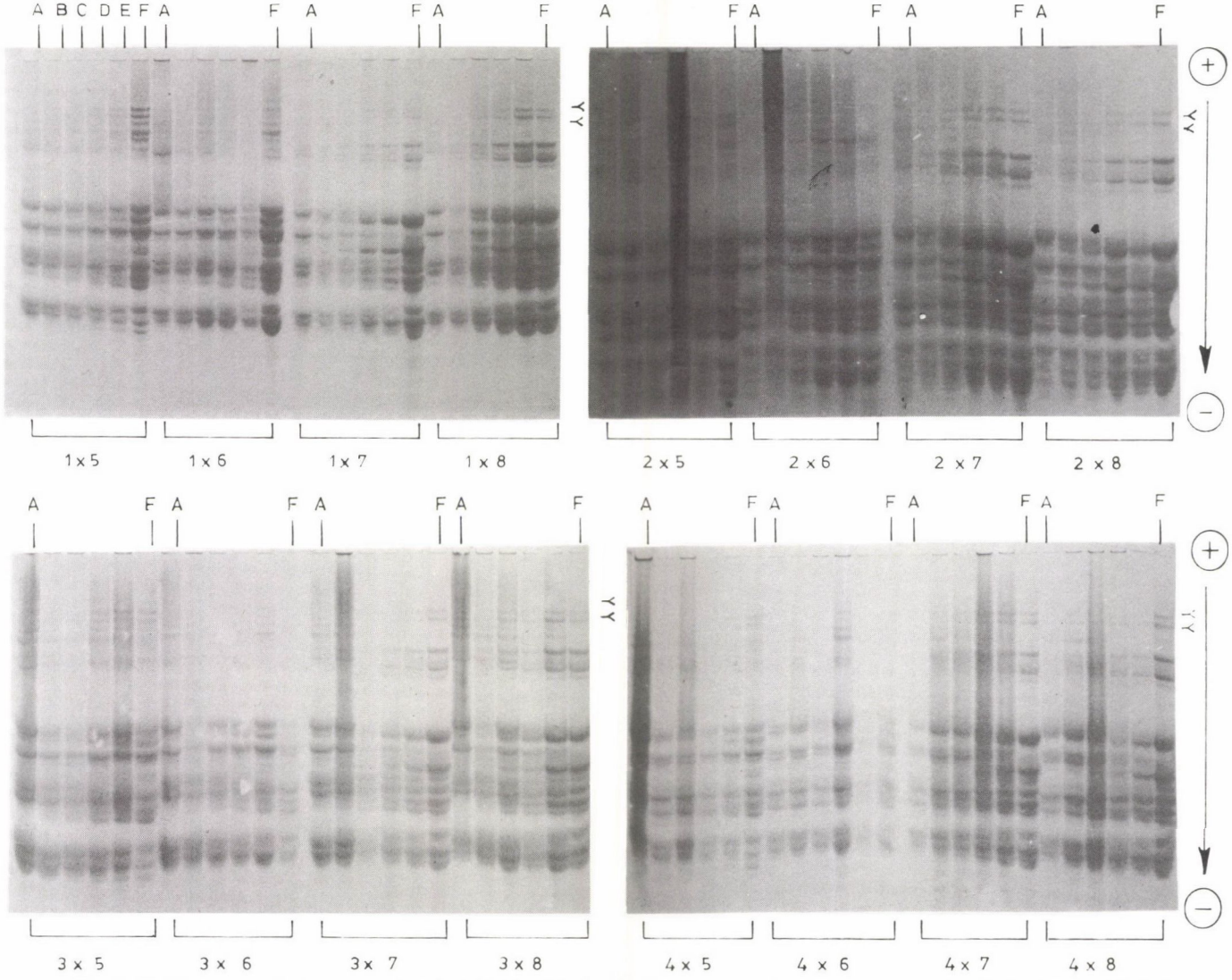

Fig. 3. PAGE patterns of gliadins extracted from blends of 4 durum wheat meals per 4 common wheat meals with 6 ratios: A: 100:0; B: 90:10; C: 80:20; D: 60:40; E: 40:60 and F: 0:100. Gel preparation and electrophoretic conditions as in Fig. 2. Durum wheat cultivars: (1) Bani Sweaf 2; (2) Bani Sweaf 3; (3) Suhag 2 and (4) Suhag 3 and common wheat cultivars: (5) G 155; (6) G 157; (7) G 162 and (8) G 165

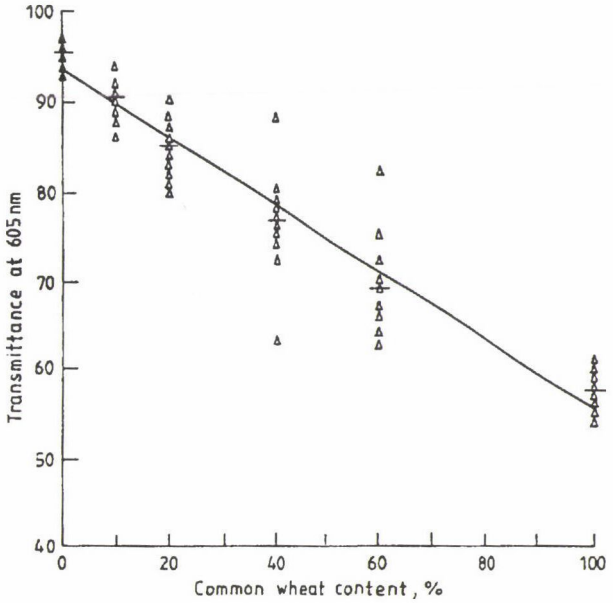

Fig. 4. Relation between transmittance at 605 nm of diluted Coomassie Blue stained slow moving bands and common wheat content in durum wheat samples. y = 93.487 – 0.378193x ; r = 0.9282***; n = 96; Δ: Values of transmittance at 605 nm; –: Average values of transmittance at 605 nm; ***: Very highly significant at P = 99.9 % probability level

Moreover, an accurate quantitative determination of common wheat or its flour in durum wheat, semolina or pasta products should be possible. Thus, this procedure is considered a useful way for determining the percentage of adulteration in durum semolina or pasta.

The continuous acetic acid PAGE system described here combines quick extraction, electrophoresis and staining procedures with good sensitivity. The thinner gels (1.5 mm) did not only decrease the amount of required chemicals, but also gave better separation. This is in full agreement with KHAN and co-workers (1985). In addition, the protein bands in the thinner gel probably has greater sensitivity to stain and the gel background is easier to destain than in the thicker one.

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Short communication

LISTERIA MONOCYTOGENES ISOLATION FROM FOOD IN HUNGARY

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For the sake of the prevention of listeriosis spreading via food, the authors performed examinations of such foods which might be contaminated by *Listeria*.

Out of 326 samples, coming from food shops and restaurants in Budapest, 12% proved to be contaminated by *Listeria monocytogenes*.

The identification of the isolated strains were performed – after 4 and 24 h incubation at 37 °C with rapid API 20 STREP, API 20 STREP and API CORYNE – with the use of ATB EXPRESSION microbiological automate.

Among the food products investigated 39.1% of raw meat products, 15.2% of heat-treated meat products, 13.6% of confectioner's and sweets industrial products, 5.5% of buffet meals and 3% of milk products proved to be positive.

Keywords: *Listeria monocytogenes*, foodborne pathogens, outbreak, food, rapid API 20 STREP, API 20 STREP, API CORYNE, ATB-EXPRESSION, GMP, GLP, HACCP

The number of foodborne diseases is considerable. Among microbes causing infection also *Listeria monocytogenes* appears (RALOVICH, 1984, 1989, RALOVICH et al., 1971; ROCOURT, 1994; WHO, 1992).

The 0.5–2 μm × 0.4–0.5 μm Gram positive coccobacillus was described first by Hölphers (1911, Sweden) with the name *Bacillus hepatitis*, which caused hepatitis in rabbits. As a human pathogen, it was isolated first by Dumon and Cotoni (1918) from the liquor of a soldier with meningitis (MINOR & VÉRON, 1984).

The first person giving detailed description of this pathogen by the name of *Bacterium monocytogenes* was Murray (1924) who isolated it from diseased mice (KOVÁCSNÉ-DOMJÁN, 1991).

In 1927 Pirie isolated it from the liver of African mice and named the microbe *Listerella hepatolytica*. It was also isolated by Gill (1929, New Zealand) from sheep and was called *Listerella ovis*. Nyfeld (1929) observed human mononucleosis syndrome and named the isolated strain *Bacterium monocytogenes hominis*. Burn

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(1933) showed it responsible for perinatal infection and finally Pirie (1940) named it as *Listeria monocytogenes* (MINOR & VÉRON, 1984).

Attention was called to the danger of this pathogen by Reiss and co-workers (1951) – *Listeria* caused neonatal granulomatosis, it was isolated from newborns in cases of neonatal pseudotuberculosis. It was supported also by descriptions of epidemics and by mortality data (MINOR & VÉRON, 1984).

It became known that *Listeria monocytogenes* occurs in soil, on the surface of plants, in silos, in animal and human faeces. It may contaminate foods often isolated from milk and meat products, from sea foods, from vegetables and from environmental samples (RODLER & KÖRBLER, 1989; KISS & RODLER, 1994).

In the course of great epidemics of the recent years, foods responsible for disease became known, too. The consumption of the following foods caused Listeria outbreaks, for example: cabbage salad in Canada (1981), pasteurised milk in Massachusetts (1983), cheese in California (1985), cheese in Switzerland (1983, 1987), paté in England (1991), jellied smoked tongue and meat paté in France (1992, 1993). Foods contaminated with Listeria show the hygienic responsibility of the handling persons (inadequate heat-treatment contamination occurring during further treatments) (RALOVICH, 1991).

The conditions of storage are also important as Listeria may multiply even at 4 °C and survives deep freezing.

These data called the attention of the authors to the introduction of the detection of *Listeria monocytogenes* from food.

Examination started with such types of food samples which were often contaminated by this foodborne pathogen according to the literature data (DOYLE, 1989; JOUVE, 1994; KEMENES, 1973; KISS & RODLER, 1992; KISS et al., 1993).

Authors wish to call attention to special risk groups – pregnant women, newborns, small children, elderlies and immunosuppressed patients – from the aspect of listeriosis.

1. Materials and methods

1.1. Sampling

Samples were taken from places where *Listeria* contamination most probable might occur (MCLAUCHLIN, 1990a, b; MORRIS & RIBEIRO, 1991; NOTERMANS & TEUNIS, 1994; RODLER et al., 1992; SZARKA et al., 1990; VARGA & KUCSERA, 1989; WILHELMS et al., 1991). Alltogether 326 food samples were taken in food stores, buffets, salad bars and various catering institutions.

The distribution of the samples was the following: 112 heat-treated meat products (55 brawns, 22 liver sausages, 18 hamburgers, 14 sausages, 3 hams); 23 raw

meats (7 minced meats, 8 hamburgers, 8 sausages); 57 sweets (31 pastry with cheese-, butter-, cream-filling, 15 ice creams and parfaits, 9 creams, 2 chestnut puree and cocoa powder); 55 buffet meals (31 salads, 5 mayonnaises, 5 garnishments, 3 filled eggs, 2 pastries with cheeses, 4 creams with mushroom-, butter-, egg-stuffing, 3 mayonnaise eggs, 2 sandwiches); 67 milk and milk-products (15 soft cheeses, 13 "half-hard" cheeses, 1 hard cheese, 4 sheep cheeses, 8 cottage cheeses, 13 curd cheeses, 4 raw mixed milks, 4 whipped creams, 1 butter cream) and 12 environmental samples (2 mincers, 2 carving machines, 2 hamburger mixing bowls, 4 trays, 2 fridges) (KISS, 1994).

1.2. Preparation

Samples were stored at −20 °C, processing was made after defrosting. Resuscitation was performed for 45 min at room temperature and then homogenization was done with stomacher (FARBER, 1989; KÖRBLER & RODLER, 1990; LÁNYI, 1980).

1.3. Enrichment, cultivation

25 g sample, inoculated into two types of enrichment broths (225 ml) were incubated at 30 °C. On the third and seventh days, spreadings were made on Columbia agar (Bio Mérieux) (completed with 5% blood) and on Forray Agar. Incubation was performed in "Generbox microaer" (Bio Mérieux) for 24 h at 30 °C.

Two enrichment broths were used:

Enrichment medium I. Soya pepton (HUMAN) 3 g; Tripton (OXOID L−42) 5 g; Yeast extract (OXOID L−21) 5 g; Corbovin^R (HUMAN) 5 g; D-glucose 2.5 g; NaCl 5 g; KH_2PO_4 1.4 g; Na_2HPO_4 $2H_2O$ 12 g; distilled water 1000 ml. After autoclaving at 115 °C for 20 min, it was completed with Tripaflavin 25 μg ml^{-1} nalidixic acid 40 μg ml^{-1} (pH 7.3).

Enrichment medium II. Brain-heart infusion (Bio Mérieux) after autoclaving at 115 °C for 20 min CNA (Bio Mérieux) was added (CNA: Colimycin 1 mg, nalidixic acid 1.5 mg per 100 ml broth).

Forray agar. Corbovin^R (HUMAN) 5 g; Tripton (OXOID L−42) 5 g; Yeast extract (OXOID L−21) 5 g; NaCl 5 g; Agar (OXOID L−11) 13 g; distilled water 950 ml – after autoclaving at 115 °C for 20 min – tripaflavin, nalidixic acid and the 5% blood were added before pouring the plates, pH 7.3 (KISS et al., 1993).

Streaking of beta-hemolyzing catalase positive colonies were performed using Columbia (Bio Mérieux) and PALCAM (Merck) agars; and the plates were incubated in "Generbox microaer" atmosphere for 24 h. Examination of the motility of strains took place in semisolid motility agar.

For the Camp test Columbia agar (Bio Mérieux) was used.

Semisolid motility agar. Nutrient broth 1000 ml – Lab-Lemco powder (OXOID) 10 g; pepton 10 g; NaCl 5 g; distilled water 1000 ml – Agar (DIFCO) 5 g; pH 7.4.

Motility was examined at room temperature, after 48 h umbrella-like figure appears in the tube.

1.4. Identification

The identification was carried out according to the API technique, with reagents, described in the manual. The reading of results and their evaluation was given by the computer.

The identification of the isolated strains were performed – with rapid API 20 STREP, API 20 STREP and API CORYNE tests – with the use of ATB EXPRESSION microbiological automate.

The biochemical reactions performed with these tests were the following: VP (acetoin production), HIP (hippurate, 85%), ESC (β-glucosidase, 98%), LAC (lactose, 63%), TRE (trehalose, 90%), β-HEM (β-hemolysis, 90%), PYZ (pyrazinamidase), GLU (glucose), MAL (maltose), CAT (catalase) positive, PYRA (pyrrolidonyl-arilamidase), α-GAL (α-galactosidase), β-GUR (β-glucuronidase), β-GAL (β-galactosidase), PAL (alkaline phosphatase, 3%), LAP (leucine arylamidase), ADH (arginine dihydrolase), RIB (ribose, 12%), ARA (L-arabinose, 2%), MAN (mannitol, 1%), SOR (sorbitol), INU (inulin, 1%), RAF (raffinose, 3%), AMD (starch, 49%), GLYG (glycogen, 4%), NIT (nitrate), URE (urease), GEL (gelatine), XYL (xylose), SAC (sucrose) negative.

The distribution of positive reactions of the tests applied are shown in Table 1.

2. Results and discussion

Out of 326 samples 39 proved to be contaminated with *Listeria monocytogenes*.

Listeria monocytogenes positivity of the examined samples was 12% average – in the following distribution: 15.2% of heat treated meat products (brawn 23.6%, liver sausages 13.6%, ham 33.3%); 39.1% of raw meats (minced meats 14.2%, hamburgers 75.0%, sausages 25.0%); 13.6% of sweets industrial products (pastry with cheese, butter, cream 22.5%, ice cream, parfait 6.6%); 5.5% of buffet meals (pastry with cheese 100.0%, mayonnaise eggs 33.3%, sandwich 50.0%); 3.0% of milk products (whipped cream 25.0%).

Listeria monocytogenes could not be isolated from environmental samples (2 mincers, 2 carving machines, 2 hamburger mixing bowls, 4 trays, 2 fridges). From

90% of the samples *Listeria* was isolated after three days incubation in the enrichment broths.

The aim of our examinations was the detection of the presence of the microbe only. Quantitative determination (colony forming units or MPN /most probable number/) was not carried out.

Table 2 shows the examined types of samples and those samples which proved to be positive.

Table 1

Distribution of the positive reactions of the examined Listeria *strains with API 20 STREP and API CORYNE systems in percentages*

VP	Acetoin production	100%
HIP	Hippurate	85%
ESC	beta-glucosidase	98%
PYRA	Pyrrolidonyl-arilamidase	0%
αGAL	alfa-galactosidase	0%
βGUR	beta-glucuronidase	0%
βGAL	beta-galactosidase	0%
PAL	Alkaline Phosphatase	3%
LAP	Leucine arylamidase	0%
ADH	Arginine dihydrolase	0%
RIB	Ribose	12%
ARA	L-Arabinose	2%
MAN	Mannitol	1%
SOR	Sorbitol	0%
LAC	Lactose	63%
TRE	Trehalose	90%
INU	Inulin	1%
RAF	Raffinose	3%
AMD	Starch	49%
GLYG	Glycogen	4%
βHEM	beta-hemolysis	90%
NIT	Nitrate	0%
PYZ	Pyrazinamidase	100%
URE	Urease	0%
GEL	Gelatine	0%
GLU	Glucose	100%
XYL	Xylose	0%
MAL	Maltose	100%
SAC	Sucrose	0%
CAT	Catalase	100%

Table 2

Distribution of the examined samples contaminated by Listeria monocytogenes

		No. of samples	L.m.		%
1.	Heat-treated meat products	112	17		15.2
	brawn	55	13	(23.6)	
	liver sausages	22	3	(13.6)	
	hamburger	18	0		
	sausages	14	0		
	ham	3	1	(33.3)	
2.	Raw meats	23	9		39.1
	minced meat	7	1	(14.2)	
	hamburger	8	6	(75.0)	
	sausage	8	2	(25.0)	
3.	Sweets	57	8		13.6
	pastry with cheese, butter, cream	31	7	(22.5)	
	ice cream, parfait	15	1	(6.6)	
	creams	9	0		
	chestnut purée, cocoa powder	2	0		
4.	Buffet meals	55	4		5.5
	salads	31	0		
	mayonnaise	5	0		
	garnishment	5	0		
	filled eggs	3	0		
	pastry with cheese	2	2	(100.0)	
	mushroom-, butter-, egg-creams	4	0		
	mayonnaise eggs	3	1	(33.3)	
	sandwich	2	1	(50.0)	
5.	Milk and milk-products	67	1		3.0
	soft cheese	15	0		
	"half-hard" cheese	13	0		
	hard cheese	1	0		
	sheep cheese	4	0		
	cottage cheese	8	0		
	curd cheese	13	0		
	raw mixed milk	4	0		
	whipped cream	4	1	(25.0)	
	butter cream	1	0		
6.	Environmental sample	12	0		0.0

L.m.: *Listeria monocytogenes* positive samples

In case of sweet industrial products and buffet meals samples made of different raw materials at the same time in the same buffet were *Listeria*-positive, probable due to cross-contamination (poor hygienic conditions).

In shops and buffets, where the taken food samples showed *Listeria monocytogenes* positivity, disinfectant cleaning was prescribed and the efficiency of disinfection was controlled.

The post-disinfection examination of tools (12 environmental samples: 2 mincers, 2 carving mashines, 2 hamburger mixing bowls, 4 trays, 2 fridges) did not show contamination with listeria.

3. Conclusions

The results of the examinations showed that the *Listeria monocytogenes* contamination of foods in Hungary is similar to those detected in other countries.

The fact that 15.2% of heat-treated meat products was positive (from 55 brawns 13–32.5%, from 22 liver sausages 3–13.6% were positive) and 39.1% of raw meats (from 8 hamburger 6–75.0% were positive) calls attention to increased control of the time and effectivity of adequate heat-treatment according to the principles of GMP, GLP and HACCP (Good Manufacturing Practice, Good Laboratory Practice, Hazard Analysis at the Critical Control Point) (BOERS, 1992).

Beside this, increased attention should be paid to the hygienic safety of tools, the environment and the refrigerators.

As pregnant mothers, newborns, infants and immunodeficient persons are especially exposed to risk, authors wish to call attention to the adequate time and the intensity of heat-treatment, to the importance of hygienic food storage and processing and to the risk of the possible contamination of certain foods with *Listeria*.

Last but not least authors wish to emphasize that *Listeria monocytogenes* is capable of living and even multiplicating at refrigerating temperatures, therefore the adequate separation of different foods and the frequent disinfectant cleaning of the refrigerators is essential for the prevention of foodborne diseases.

Authors will report in the future about the continuation of the work, about the serotype distribution of the isolated strains.

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BOOK REVIEWS

Aroma perception, formation, evaluation
Proceedings of the 4th Wartburg Aroma Symposium Eisenach, March 1st-4th, 1994

M. ROTHE and H.-P. KRUSE (Eds)

Eigenverlag Deutsches Institut für Ernährungsforschung 1995, 712 pages

The book covers the most important lectures of the 4th Wartburg Aroma Symposium. Highlights from the 7th Weurman Flavour Research Symposium were summarised by H. E. Nursten.

The symposium had three main topics: flavour perception, aroma formation and aroma evaluation.

On the area of flavour perception, lectures (13 presentations) focussed on nutrition psychology, role of motivation and emotion in olfactory and taste perception. Among others, anosmia, olfactory event-related potentials, objectivation of taste sensation by gustatory cognitive integration of receptor patterns, studies on flavour memory and body alcohol level, validity of the psychophysical Power Law are discussed. Psycholinguistics in aroma research, peculiarity of flavour perception in food emulsion, the effect of matrices, the synergistic and antagonistic effects between aroma compounds are also touched.

The second main topic (8 lectures) was the aroma formation, among others in dairy starters, complex cheese flavours, Brassica vegetables, sardine preserves. Other papers involve chirality of aroma compounds, synthesis and enantiodifferentiation of C13-norisoprenoid aroma compounds, alkylcyclanones as synthetic aroma substances. The third important part is the aroma evaluation. Studied products were wine, Swiss cheese, pastries of high fat content, steak, brewed coffee, raw cocoas, barley during malting, apples.

The connection of instrumental analysis and sensory perception, flavour analysis and quality assurance, new analytical technics (GC-IRMS), GC CHARM analysis were also presented. Finally the two workshops, one on the meaning of flavour and the other on fat-reduced food products, an energy intake, are described.

The book may be recommended to those who deal with aroma chemistry and sensory sciences, mainly from theoretical point of view.

M. TÓTH-MARKUS

Akadémiai Kiadó, Budapest

Environmentally responsible packaging - a guide to development selection and design

C. LAUZON and G. WOOD (Eds)

Pira International, Surrey, UK 1995, 136 pages

This book is an excellent presentation on the one aspect of burning question of our century "Environmental Protection", the impact of packaging materials on the environment.

The Chapter 1 deals with the problems and perspectives of packaging materials by providing brief statistics. Chapter 2 broadly discusses the questions associated with the identification of unresponsible packaging materials. In this chapter the proportion of various waste materials and the energy factor involved in the production of packaging materials with reference to its responsibility and irresponsibility towards environment are discussed.

In Chapter 3 the authors summarize the options to become responsible to environment. The flow chart on product packaging life-cycle would be a guide for those who are looking for new packaging materials with minimum unfriendliness to the environment. This chapter also presents possible legislative efforts to control the unresponsive packaging materials.

Chapter 4 deals with optimization of packaging technology with an aim at minimizing environmental damage. A detailed account on recycling is presented in Chapter 5 covering corporate and local governments' efforts in few countries. This chapter also emphasizes the importance of material selection for recycling. Few major recyclable materials were taken and analyzed for their successful recycling process.

Chapter 6 deals with the economics and the practical difficulties involved in the re-usage of packing materials. The authors have take PET bottles for representative analysis. Chapter 7 deals in detail with the efforts made by legislative authorities in various countries to reduce the stress on the environment by the irresponsible use of packing materials. This Chapter would be very useful for legislative planners.

In the final chapter the authors present their views for the successful designing of environmentally responsible packaging. The bibliography of this book provides quite useful background information.

This book is highly recommended for packaging industrialists, environmental managers and academicians who deal with this subject.

J. MONSPART-SÉNYI

ANNOUNCEMENT

'96 International Food Machinery Exhibition
(FOOMA '96)

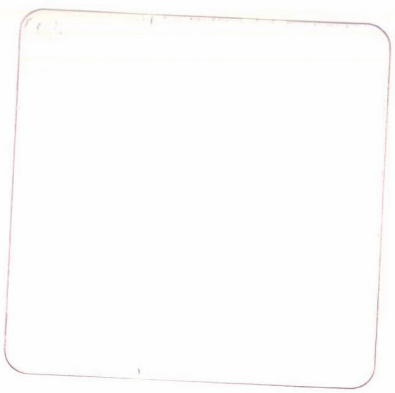
The Exhibition will be held at the Nippon Convention Center, Makuhari (Makuhari Messe), Japan, May 28 – 31, 1996.

The International Food Machinery Exhibition (FOOMA) is the largest trade show for the food manufacturing and processing industries in the Asia-Pacific Rim region.

This trade show covers a wide range of the food manufacturing and processing equipment, food materials, ingredients as well as packaging, conveying, sanitation control, system engineering and waste control equipment.

With 450 exhibitors and more than 90 000 visitors expected to attend, FOOMA '96 affords the best opportunity for you and your business.

Further information can be availed:
Secretariat of FOOMA
Toranomon Center Blg., 1 – 16 – 17, Minato-Ku, Tokyo 105, Japan



9th World Congress of Food Science and Technology Plenary lectures

Effect of acidification and fermentation on the quality characteristics of canned mung bean (*Wigna radiata Wilczec*) sprouts

CANTARELLI, P. R., NOGUEIRA, J. N., GALLO, C. R. & VERTONI, P. C.

Processing and storage effects on the quality of dehydrated apples

HEGEDUŠIĆ, V., HERCEG, Z. & REXHEPI, A.

Effects of microwave heating on the chemico-nutritional value of soybeans

ŠAKAĆ, M., RISTIĆ, M. & LEVIĆ, J.

NOTICE TO CONTRIBUTORS

General. Only original papers will be published and a copy of the Publishing Agreement will be sent to the authors of papers accepted for publication. Manuscripts will be processed only after receiving the signed copy of the agreement. Manuscripts in English should be typed double-spaced on one side of the sheet and should not exceed 20 pages.

Title. The title should be concise and informative. It must be followed by the authors' names and the address(es) of the institute(s) where the work was carried out.

Summary. The article should be preceded by a summary (not exceeding 150 words) giving a self-explanatory compendium of the essence of the paper. At the end of the summary some 4 keywords should be put in alphabetical order.

Text. The article should be divided into the following parts: *Introduction* beginning on a new page without a title containing a survey of related literature and the objectives of the work, followed by *Materials and methods; Results; Conclusions.*

Symbols. Units, symbols and abbreviations should be used according to SI (System International) rules. Unusual abbreviations must be explained in the text or in an appendix.

Figures and tables. Illustrations must be cited and numbered in the order they appear in the text. All line drawings should be submitted as clear, glossy, black and white originals or photographs of good quality. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each figure. Figures and Tables, each bearing a title, should be self-explanatory and numbered consecutively. The number of measurements (n), their mean values ($\bar{x}$) and standard deviations ($\pm s$) should be indicated. To prove the objectivity of conclusions drawn from the results mathematical statistical methods must be used. Quantitative evaluation of data is indispensable.

References. The reference list (Literature) should be in alphabetical order as follows:

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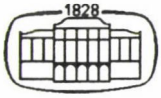

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BUDAPEST

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EDITORIAL NOTE

9th World Congress of Food Science and Technology
July 30 – August 4, 1995
Budapest, Hungary

The Hungarian Scientific Society for Food Industry (MÉTE) organised under the auspices of the International Union of Food Science and Technology (IUFoST), the 9th World Congress of Food Science and Technology between 30th July – 4th August 1995. On this occasion, 1200 food and nutrition scientists were guests in the Hungarian capital, Budapest. As Central and Eastern European markets have opened in the last four to five years and are rapidly developing, the meeting addressed not only nutrition scientists but also food technologists and the business community.

The bridge-building between East and West – symbol of the World Congress – implies the building of business connections too and the list of people attending that. Not only scientists but also representatives of multinational companies came in great numbers, people already with large holdings in Hungary and wanting to widen their markets.

The Scientific Committee of the Congress with Hungarian, Austrian, German and Polish representatives of national scientific societies has determined the main issues to cover all field of food science and technology.

This major event hosted 400 lectures in 43 symposia, 11 roundtable discussions and 8 satellite programs and more than 700 posters were presented. Around 60 countries from all over the world were represented. The topics covered raw materials for food manufacturing, modern processing technologies, nutrition science, food composition, the inspection and safety of foodstuffs, and economic and environmental effects. Abstracts of the presentations were issued, the congress concluded with 14 technical tours where participants were invited to visit Hungarian food-processing plants, to offer the possibility to have a look at their company, its environment and technology from a professional viewpoint. Great interest was shown towards NESTLÉ, UNILEVER, PARMALAT, PICK, HUNGAROVIN, EGERVIN. Multinational firms are now settled in Hungary and are manufacturing not only their usual brand name products but are also making efforts to market genuine Hungarian products.

This congress is the main event of the IUFoST's scientific programme. Held at quadrennial intervals, it provides an opportunity to discuss old and new problems in food science and technology, to focus attention on major issues, to set new goals and

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to influence the trends of future research. One of the principal objectives of IUFoST is to foster international interaction and communication at all levels.

The papers of the plenary session are presented in the following order:

1. Opening address by L. LAKOS, Minister of Agriculture
2. Founders lecture by J. F. DIEHL
3. Research program of the European Union by L. BRESLIN
4. Food and environment in the future by P. GRAY
5. Food science and industry by M. HORISBERGER
6. Sustainable development of Hungarian agriculture by I. LÁNG

P. A. BIACS

Acta Alimentaria, Vol. 25 (2), pp. 97–100 (1996)

OPENING ADDRESS OF DR. VET. MED. LÁSZLÓ LAKOS HIS EXCELLENCY THE MINISTER OF AGRICULTURE IN HUNGARY

Ministry of Agriculture, H–1055 Budapest, Kossuth Lajos tér 11. Hungary

Articles of food are of special significance all over the world bearing no substitute they often shape the destiny of whole nations. Being one of the key elements of humanity's survival day by day their value is priceless. Science plays an ever increasing part in the production of food, in the creation of new production technologies and not at last in quality control. Institutes and scientists of great prestige have taken part in research for several decades.

The host organization, the Hungarian Scientific Society for Food Industry has been participating at world congresses since 1966 and is a founder member of the World Union established in Washington D.C. in 1970. Several well-known researchers and internationally recognized technology engineers have built professional relations over several decades in order to be granted the right to organize this event and to facilitate for the best representatives of food research to meet in our country at this world scale forum.

In the scientific society having at present more than 5000 members, 200 legal entities, the whole area of food industry, thus entrepreneurial businesses, universities research institutes and quality control institute are represented.

MÉTE (Hungarian Scientific Society for Food Industry) made arrangements for the world congress for several years. The token of the congress is the Chain Bridge. This world famous sight of our capital city can be a natural symbol: bridge between different regions of the world.

Beyond the sharing of information on the findings of scientific research the main subjects of the 9th World Congress are technology transfer and innovation. Nowadays, there is a good opportunity for the flow and exchange of intellectual products in the region of Central and Eastern Europe. Scientific contacts also contribute to the renewal of economy, to domestic and foreign capital investment to the strengthening of viable businesses and to the restructuring of food production. Good scientific cooperation can have an enormous significance beyond this, in terms of closing the gap between developed and underdeveloped regions and facilitating for the developing countries to catch up.

Food production of the world features special characteristics today. There are countries where to produce staple food is a serious problem, whereas in other places

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new developments in the technology are every-day-events almost in every field and new methods of widening the variety are the most important.

The World Congress can help alleviate this tension because scientist coming from different regions can exchange their experiences, views.

We are glad to witness the great interest expressed for the World Congress. We can greet representatives of nearly 70 countries, 350 lectures, oral presentations will be made and more than 600 posters can be studied by the researchers. The schedule of scientific events is very busy: there are 43 workshops, and at 13 round table discussions can experts share their views. International organizations projects of research programs, international scientific journal publishers make use of the opportunities of the world congress - this is an excellent chance for them to meet the most outstanding representatives of the profession and talk to each other.

There can be a lively scientific life at the congress and I do hope that this will be the case. This can be promoted by the novel idea of the organizers, that is to invite you - at midterm - on Wednesday - to visit the most well known companies of Hungarian food industry, those that are pleased to be introduced on the occasion of a professional visit and are willing to show their technologies and offer a tasting of their products.

Hungarian food science based on the amalgamation of basic research and agro-technical sciences can take pride in a past history rich in accomplishments. Our first worldwide known success is associated with the name of Nobel prize winner Albert Szentgyörgyi. He extracted and verified vitamin C - this indispensable biochemical compound - from paprika grown in Southern Hungary, near Szeged. The quality of Hungarian red pepper (paprika), fermented salami (sausage), Tokay wine, goose liver, honey, fruit juices, excellent fresh vegetables is proven by scientific research, justifying that our goods are popular for this reason in a number of markets.

Present results of Hungarian scientists dealing with food research are also remarkable. In international scientific journals we could read about their results in improving the investigation method of food composition, and the practical implementation of basic knowledge of physics, chemistry, biology. Research is promising especially in the field of non-destructive analysis, ionizing radiation and the application of fermentation procedures. Now we can say that food producers and processors rely significantly on science in Hungary.

This fact is important because in our country food production and processing are among the most important sectors of economy. Our agricultural conditions are good. There is some form of agricultural production ongoing on about 70% of our country's territory. This proportion has been due to the favourable ecological conditions. In our country arable land and horticultural cultivation, production of seeds and animal breeding are the main lines of activity. This is the basis for the processing industry

which is regarded as increasingly up to date against the background of international comparative date. There I would like to mention that I was pleased to hear about the participation of CIAA – the Confederation of the Food and Drink Industries of the EEC at the World Congress. This significant international organization has been invited by ÉFOSZ – the Federation of Hungarian Food Industries. ÉFOSZ has been given an observer status in the in CIAA, lately. I wish you a lot of success in your probably mutually beneficial cooperation.

Hungarian agriculture produces 16–18% of GDP. The share of food industry is one third – one fourth of this. Agriculture contributes 25% of the country's export revenues. Every 5th employee is engaged in food manufacturing but at least half of the active population takes part in agricultural production in one form or the other.

In the last few years the Hungarian agricultural – food industrial production has declined by almost a third due to some negative processes hitting our economy. It seems however that agricultural production in our country has kept its flexibility in adjusting and this provides for the creation of conditions of our accession to the EU, and for the increase of our production, and our exports at the end of the year.

The Hungarian government has done its utmost and the measures planned also serve this end. At this forum I should emphasize in particular those measures that we have in the spirit of a new quality policy, the protection and safety of consumers. These are aimed at to encourage the quality improvement, too, to have stricter control and to improve the efficiency of regulation.

I am glad that the members of the Hungarian Academy of Sciences have been invited to attend the World Congress as well as other famous representatives of Hungarian scientific life, professors of universities and research institutes. A strong relationship with agriculture, a change of varieties, plant improvement and reliance on the results of animal breeding have generated a vivid scientific cooperation in the Hungarian food economy. These are manifested in the competitiveness of food sold in domestic and foreign markets. Emphasis has shifted towards free trade in our country and in Central and Eastern Europe as a whole. Having recognized this, a new Food Act taking those mentioned above into consideration is being drafted. Hungarian food legislation also relies on rules justified by science. The Hungarian Food Book is being prepared. It will supply information to raw material producers, processors, tradesman and consumers.

The good reputation and international competitiveness of Hungarian food have been due to the excellent primary materials, plant varieties and animal species making also use of Hungarian soil and climatic conditions, but also harmless and modern methods of processing and lately updated packaging formula have also contributed to that. Our country's food technologists make significant efforts to renew technologies

of processing, to utilize additives and auxiliary materials in an expedient and economic manner, and to modernize this industry. Our food articles get to many countries of the world by various channels of trade and are popular among old and new customers.

Our country is a member of several international institutions, like FAO and WHO. The representatives of these UN specialized agencies are not only present here but have taken an active part in organizing scientific symposia.

It is a special honour for us to see so many representatives of EU member states at the World Congress. At the plenary session well known experts from the European communities will share information with us about their policy on European food research, development, and closely related environmental aspects.

We are glad to welcome all those endeavors of integration and harmonization which open up for our country new avenues of communication on the basis of reciprocal interest and facilitate our catching up with the industrially advanced countries.

I am convinced that the majority of common problems of mankind can be resolved on the basis of cooperation of peoples – of course also academics and politicians – far more efficient than ever before. One of the most important global task is the mapping of potential new directions of food production, research of new forms of innovation.

A lot depends on you – on the activity of the World Congress. In this spirit I would like to wish you a very successful Congress. With these words I declare the Congress open.

Acta Alimentaria, Vol. 25 (2), pp. 101–104 (1996)

FOUNDERS LECTURE

FROM WASHINGTON 1970 TO BUDAPEST 1995 – TWENTY-FIVE YEARS OF IUFOST*

J. F. DIEHL

Karlsruhe, Germany

Although plans to form an International Union of Food Science and Technology date back to the early 1960s, the Union was inaugurated at the International Congress of Food Science and Technology held in Washington in 1970. This year's Congress in Budapest therefore marks the 25th anniversary of the Union – a good opportunity to look back and see what has been achieved.

The motto of the Washington Congress was *The Science of Survival – SOS/70.* Two years earlier Paul Ehrlich had published his famous book The Population Bomb in which he stated that food production had fallen behind world population growth. He predicted that in the 1970s hundreds of millions of people were going to starve to death. He foresaw "the greatest cataclysm in the history of man". Although many scientists considered Ehrlich's frightful scenario exaggerated, the prediction was generally accepted that world population would double by the end of the century, and that agricultural production could not keep pace with this increase. At the Washington Congress food scientists were called upon to develop better methods of food preservation, so as to minimize spoilage losses. Another important role for food scientists was seen in the development of processes for the production of food from unusual sources, in particular proteins from leaves, algae, yeasts, and bacteria.

Fortunately, Ehrlich and the many others who wrote similar books and articles were wrong. The predicted calamities did not occur. In the contrary, life expectancy has increased dramatically, even in the poorest countries. Two factors were mainly responsible for this favorable turn of events: Thanks to birth control, world population has grown less than expected. Instead of the 7 billion predicted in 1970, world population in the year 2000 will be 6.1 billion. On the other hand, food production has increased marvellously, thanks to the Green Revolution.

* The author has submitted the full length, fully referenced text to LWT, the official journal of IUFOST.

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Hunger has not been eliminated. About 800 million people in developing countries still do not have access to enough food to meet the basic daily needs for nutritional well-being. However, the problem is not a lack of food in world markets, but inadequate distribution and poverty. To the dismay of farmers and Agriculture Ministries all over the world, food prizes are very low – which is the best indication that there is no shortage.

The large resources that went into development of single cell protein and other types of novel proteins during the 1970s were almost completely wasted. There is enough low priced protein produced by agriculture and fisheries, so that novel proteins had almost no chance. Great efforts have also gone into developing and perfecting new techniques of food preservation, food irradiation in particular. This technique is available – but the food industry is making very little use of it. Contrary to what we hoped and expected around 1970, food science and technology did not play a central role in the fight against hunger. The credit for the improvement of the situation primarily goes to agriculture, especially to plant genetics.

Another important issue at the Washington Congress was chemical contamination of food. In the 1960s and 70s this seemed to be a problem as grave as the expected shortage of food. Rachel Carson had published Silent Spring in 1962, a world best-seller which claimed that mankind was being progressively poisoned by food contaminated by chemical pesticides and additives. Carson thought only man could produce cancer-causing chemicals, and man created his own "cancerous universe". Also in the 1960s news came from Japan about mass poisonings caused by mercury (Minamata disease), cadmium (itai-itai disease), and polychlorinated biphenyls (yusho disease). The fear grew that perhaps all over the world people were threatened by chemical contamination of food. At the Washington Congress several speakers pointed out how little was known about food contaminants; food scientists were challenged to do their part to avoid the catastrophies predicted by Carson.

Tremendous progress has been made in this area – and our profession has played a most important role in this. Food scientists have improved the methods of food analysis to an extent that we are now speaking of nanograms and picograms where we used to deal with milligrams. Monitoring programs exist in most countries. We know the daily intakes of all important food contaminants. Food technologists have also done their part. Canning, for example, is now achieved without lead migrating from the solder into the food. Modern smoking processes permit production of smoked foods with minimal levels of benzopyrene. Much less sulfite is used in food processing than in the past. Curing of meat is done with less nitrite. Beer, surprisingly, turned out to be an important contributor to the nitrosamine intake, and improvements in the technology of malt drying have lowered average nitrosamine intakes to one third.

Many other examples of such improvements could be given. As far as chemical contaminants are concerned, our food is safer today than ever before.

Carson deserves credit for calling attention to real problems. The massive use of DDT and other persistent pesticides had to be stopped. But she was wrong on several counts. Natural products can cause cancer as effectively as synthetic chemicals, and the idea that cancer incidence could be lowered by abolishing food additives has received no support. Tremendous resources went into the search for carcinogenic substances in the 1960s and 70s, with very little success. A complete change of thinking has occurred since then. In 1981 Doll and Peto presented epidemiological evidence that diet was indeed an important cause of cancer – but not contaminants in the diet, not food additives. Overeating (obesity), too much fat, too much alcohol are today considered as important factors in carcinogenesis. The former search for cancer-causing substances has been replaced by the search for cancer-preventing food constituents, such as dietary fiber and antioxidants.

The work of Bruce Ames and his collaborators, who have shown animals and humans to have numerous defenses against toxins that make them very well buffered against low doses of almost all toxins, whether synthetic or natural, has much contributed to this new thinking. Unfortunately, however, this change of thinking in the world of science has not yet been noticed by many consumer activists, journalists and politicians who still fight the "War on Cancer" by condemming food additives.

In countries where the food supply is plentiful, a critical trend in consumer behavior was apparent 25 years ago, and food acceptance was another issue that received much attention at the Washington Congress. The problem is as acute today as it was then. Books on food and nutrition addressed to the general public are frequently sensational, misleading, and demonstrate an anti-industry, anti-science, anti-technology bias. Newspapers, magazines and television are similarly critical of today's food supply. Derogatory remarks about "industry food", "junk food", "empty calories" often culminate in the recommendation to buy unprocessed, organically grown food.

George Stewart, the first President of the Union, has committed IUFOST "to serving mankind in an area of critical need – providing safe, stable, palatable, and nutritious food". Yet, on the consumer side, our profession's image often does not correspond to these noble goals. What can be done to change this situation? The food industry is not much help. Labelling such as NO PRESERVATIVES ADDED implies that preservatives are harmful. The industry leans over backward to present products with labels containing the words bio, land, farm, original, home etc. to create the impression that this product has never gone through the gates of a food processing plant. And are we not also inclined to minimize our activities when we face the public?

Minimal processing is the catch word. Does it not imply that processing is undesirable?

It appears to me we food scientists are not adequately trained to handle the problem of consumer acceptance by ourselves. We have not learned to deal with arguments based more on emotion or ideology than on facts. We need help from our colleagues in the humanities. How about organizing a joint workshop or symposium of IUFOST and IUPsyS, the International Union of Psychological Sciences?

Moreover, I think we should more actively seek contact with consumer representatives. Many of them simply lack information. I know there are those who don't want our information ("Stop bothering me with your facts! My mind is made up"). But there are enough others who would be interested to find out what food technologists and food engineers are actually doing. Perhaps IUFOST could suggest a joint workshop to IOCU (International Organization of Consumer Unions). Perhaps IUFOST shortcourses on food science and technology could be offered to journalists. I have not only IUFOST or its Executive Committee in mind when I suggest that the public image of food science and technology needs improvement. All of us can contribute to this in our own individual professional sphere.

Some other major topics discussed at the Washington Congress are as important today as they were then. However, time permitted me only to discuss three issues that had received much attention 25 years ago: Issue no. 1, hunger in the world, is still a problem of central importance, even if it has not grown into the cataclysmic dimensions predicted at that time. The fact that the worst has been prevented was due to progress in agricultural productivity and in birth control, whereas the contribution of food science was small. Issue no. 2, chemical contamination of food, although it still causes occasional headlines, has become a non-problem in most parts of the world. Food science and technology have played a most important role in this encouraging development. Issue no. 3, food acceptance, or perhaps more accurately, the acceptance of modern food science and technology by the general public, is as acute today as it was in 1970, and leaves much for us to do.

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RESEARCH PROGRAM OF THE EUROPEAN UNION

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Looking at the programme of presentations, symposia, round tables, this congress will cover a tremendous range of topics within food science and technology and the proceedings coming forth will be a vital benchmark for the state of the art of food science and technology and where we should concentrate in the future. In the Commission, we shall take careful note of the various recommendations coming forth, which will be of tremendous help to us in design of future programmes for food research.

During 1994, we succeeded to have the Fourth Framework Programme adopted with a 5-year duration from 1994–1998, with a budget of 12.3 billion ECU. This is a major vote of confidence in European Research and Development to deliver to society and industry and has the full support of the European Council, Parliament and the Commission.

Life Sciences – incorporating Biomedicine, Biotechnology and Agriculture, Fisheries and Food Processing – have received a strong vote of confidence, with 13% of the total funds.

On 26 November 1994, after a passage through Council and the European Parliament, we succeeded in having adopted the new specific programme for research, technological development and demonstration in the field on agriculture and fisheries (including agro-industry, food technologies, forestry, aquaculture and rural development). This programme, running from 1994–1998 will have a funding 607 MECU (with possibilities for top-up monies later).

The key aspect of this programme is that rather than having separate and independent programmes on agriculture, forestry, fisheries, food science and technology or non-food technologies, all these fields are brought together to interact in one large programme.

We will also be looking for considerable interactions between programmes, e.g. between Biotechnology, Biomedicine and Agriculture and Fisheries.

If we focus a little on the Agriculture and Fisheries Programme, the key criteria for eligibility are:

0139-3006/96/$ 5.00

- Scientific and technical excellence and novelty
- Pre-competitive
- Transnational collaboration
- Potential exploitability
- European dimension
- Private sector participation.

Within the programme, we have identified 5 main areas, where projects will be funded:

1. Integrated Production and Processing Chains 15%
2. Scaling-up and processing methodologies 7%
3. Generic Science and Advanced Technologies for Nutritious Foods 16%
4. Agriculture, Forestry and Rural Development 37%
5. Fisheries and Aquaculture 17%

We also have some new areas, viz.

- Concentration 8%
- ELSA 1%
- Training 5%
- SME's up to 10%

You will be aware that we are placing considerable emphasis on the non-food area (areas 1 & 2) and already we see great potential in the areas such as oilseeds, cereal starch, secondary metabolites of plants and microbes, plant and tree fibres. It is very likely in future we will see more food/non-food interactions.

Turning to the food science part of this programme, we are building on the experience we have gained from previous programmes, especially:

- Many COST actions where the first true European networks of food scientists were put together;
- Later the FLAIR Programme (1988–1993), where some very important concerned actions (e.g. in Sensory Analysis, Spectroscopy, Dietary Intake Patterns) were put in place;
- Finally, the AIR Programme (1991–1994), which was the first of the integrated programmes put in place. This formed part of the Third Framework Programme. Within this programme there was an expansion of the food area with a total of 72 projects funded, with EC contribution of 57 MECU (out of a total of 330 MECU).

Within the new FAIR programme in the Food Sector, we are aiming to focus on the following main priorities:

- Consumer Nutrition and Well Being;
- New and Optimized Food Raw Materials;
- Advanced and Optimized Technologies and Processes;
- Generic Food Science.

We have recently had a call for proposals, which resulted in over 900 proposals from all over Europe (of which 120 were in the food sector).

Following evaluation of proposals in the area of nutrition and new food materials, we are proposing to fund a first series of food proposals to the value of 16 MECU.

What we have noted from the response to the first call is:

- In the nutritional field, there is a tremendous potential with teams from nutritional science, medicine, biochemistry, processing technologies working with industry on areas such as polyunsaturated fatty acids, complex phenols, anti-oxydants, flavenoids – linking these with human health and incorporation into goods.
- We also see potential in applying biotechnology and traditional breeding methods to produce plant varieties, which are safer or with special nutritional benefits.

This area of food nutrition has been identified as an area of special importance in this programme and this was one of the key recommendations coming from the European Science and Technology Assembly, which was established by our previous Commissioner for Science and Research, Prof. A. Ruberti. Their view was that Nutritional Science needs some re-establishment in Europe – in many countries, it has been in decline. We will make efforts to fund substantial projects in this area and also to use our training grant funds to bring fresh blood into this area.

It was interesting also to note that in all the successful projects, there was direct industry involvement, not just the food industry, but also industries such as pharmaceuticals. In this field also, there is great potential for N–S partnerships.

Our next call will close on 15 September and this will focus on existing and advanced food processing technologies and fundamental food science.

In food technologies, we will continue to fund innovative applied projects in fields such as dairy, meat, cereals, fruit and vegetables technologies, but we are also placing quite an emphasis on the development of advanced technologies, which can be applied by the food industry in the years to come. We are particularly interested in

milder processing technologies, non-thermal treatments, combinations and especially use of robotics and automation technology.

This call will also contain more fundamental areas such as biochemistry, biophysics, biotechnology, toxicology and food microbiology.

Our experience with the recent programmes in the food area is that there are now excellent European networks established in many areas of food science. What we would like to see happening is increased sharing of expertise and resources between laboratories throughout Europe. It is also encouraging to see in many of these shared cost projects - European food industries, especially the larger ones - collaborating together in pre-competitive research. This can only be to the benefit of European food science and ultimately to the benefit of our European industry, which is one of the key aims of our programme.

We are also noting in the food area a much increased number of applications from our young scientists, who are applying for research bursaries - and are quite prepared to move to another country for 1–2 years. Their chosen subject areas of study suprise us with the high level of excellence. We have also provided help and funding to the new European Masters Degree in Food Studies, which should commence this autumn.

We are making in our new programme a particular effort to bring more food and non-food SME's into research projects. It has been difficult in the past to convince SME's of the benefits of participating in research projects. We propose to set aside up to 60 MECU to help European SME's to prepare proposal, find partners and to participate in co-operative medium tech research projects. We are already receiving quite a number of proposals and we have a first selection of these projects ready for funding. No deadlines apply to these proposals so SME may submit at any time.

This is the first programme in which the 3 new Member States of Austria, Sweden and Finland are participating with full funding rights. Iceland and Norway have similar rights.

These countries in the first round of proposals are coming forward very strongly and it seems their researchers and industry have prepared themselves very well. In the Agro-Industrial area of the programme, we view them with particular potential and expertise in the non-food areas of biomass, green chemicals and polymers and in wood technologies. In food, they have been very strong in the food nutrition sector.

Countries from Central and Eastern Europe and also Switzerland can participate in projects but will not receive funding from the programme.

For Central and Eastern European countries, there is also the opportunity to participate in COST actions, many of which are underway in aspects of food

technologies. Also, there will be a special call for Central and Eastern Europe launched this autumn, a type of follow-on to COPERNICUS.

Finally, we are anxious to disseminate the broad results of our projects to as wide an audience as possible. For a number of years now in the Food area, we have the very effective FLAIR-FLOW action, which is disseminating results every month in a user friendly way, in 17 different countries. We have launched a similar action last year for the non-food area called AIRID, which is now starting up.

This is an overview of what is happening in the European Commission in the area of food R & D. We are making, I think, good progress and soon, we will be thinking towards the year 2000 and designing the 5th Framework Programme.

The reports and outcome of this World Congress of Food Science and Technology have the potential to be of tremendous scientific importance to us as we start our work on our next Framework Programme, so I would urge workshops and symposia to come forward with recommendations for future priorities.

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FOOD AND ENVIRONMENT IN THE FUTURE

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Throughout the world in both primitive and industrialized societies the major impact of mankind on the natural environment has arisen through food production. The industrialization of agriculture has increased pressure on natural resources to the point where farming systems have clearly become non-sustainable. On the other hand pollution arising from industrial activities is affecting the quality of natural resources and could well be a limiting factor in the production of an adequate supply of wholesome food for the world's population.

This paper examines these questions broadly but not exhaustively, firstly analyzing the food demand/supply relationship and then drawing the conclusion that a fundamental change has already occurred in this relationship due to the exhaustion of the possibility of increased efficiency in production to keep pace with even modest predictions of population growth.

Natural resources are themselves under pressure and in many cases are being used unsustainably, as is shown by the results of recent European Community research on water resources. Although the debate on climate change, its extent and possible consequences continues to rage the evidence that there is an unprecedented increase in greenhouse gases arising from human activities is incontrovertible. The International Panel on Climate Change has concluded that anthropogenic change is occurring and this, in turn, will lead to effects on agricultural production that are, as yet, unpredictable.

The complexity of biogeochemical cycles in the environment and their potential to transport contaminants into the food supply is examined. The importance of airborne transport is illustrated with reference to recent EU research in the Mediterranean. Ice transport mechanisms in the Arctic are explained to illustrate the need to take a global approach. The European and Asian Arctic is a region with large economic potential. Release of ice borne contaminants takes place at the ice edge spring melt of Greenland which is a nursery area for North Atlantic fish and this could place yet one more stress on beleagured fish stocks.

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Finally the case of cadmium pollution is used to illustrate the need for control at source as the only effective method of preventing undesirable levels occurring in food.

1. The problem

In 1798 in his "Essay on the Principle of Population, As It Affects the Future Improvement of Society", Thomas Malthus, the gloomy parson, stated that "The power of population is indefinitely greater than the power of the earth to produce subsistence for man." He argued that world population would expand exponentially whilst world food supply would increase in a linear fashion. Since population would always exceed the food supply, humanity was destined to a miserable lot of continuous, marginal starvation. In the intervening period the developing world was being explored and exploited rather than developed, whilst in the Western World the industrial revolution led to a mechanisation of farming and crop yield grew at such a rate that Malthus' predictions remained unfulfilled.

Over 200 years later in the Club of Rome report "Limits to Growth", MEADOWS and co-authors (1974) set out a picture of a starving world expanding both its population and its resources at a rate that was destined to result in economic collapse through scarcity, pollution, famine, plague and societal tension leading possibly to armed conflict. These predictions have been discounted by various critics who rely more on uninformed optimistic extrapolation of the past than soundly based economic or scientific analysis. The continued prosperity and expansion of agricultural production in the '70s and '80s is cited by them as evidence that the scenario set out by the Club of Rome is invalid. It is the author's view that a systematic examination of the world economy and the environmental situation shows a number of indicators that are signalling that a turning point has been reached. Furthermore climate change is likely to aggravate a situation that even on a "business as usual" scenario would be untenable.

It is not possible in one paper to exhaustively examine all the factors relating food supply and the environment. This paper will, therefore, examine some aspects in detail in order to illustrate the complexity of the problem and the need for fundamental research and far sighted policy making. The relationship will be examined under three headings:

- The demand/supply situation;
- Some environmental production limiting factors;
- Some quality limiting factors of environmental origin,

and the complexity of the processes involved will be highlighted by the description of some unexpected phenomena in each area.

2. The growing demand for food – the sustainable limit

The yield of agricultural crops is basically determined by the amount of light the plant receives for photosynthesis and nutrient and water supplied during the growing season. During historical times, apart from short term variations, the climate has changed very little and important changes in yield were brought about by human intervention. Even before agriculture became industrialised there were continuous improvements in productivity arising from such techniques as plant variety selection, improved irrigation and crop rotation. Nevertheless, even during this period there are ample examples of soils being rendered infertile by inappropriate methods of husbandry, leading in extreme cases to desertification.

In the second half of this century increased efficiency in agriculture from such factors as mechanisation, improved plants hybrids and increased use of fertilisers and plant protection products has enabled food supply to keep broadly in step with an increasing growth in World population. BROWN (1994), in his paper 'Facing Food Insecurity', argues that the 'per capita' production of grain is a good indicator of the state of the World demand/supply situation for food. From 1950 to 1984 total grain production increased at an average annual rate of 2,3% but since 1984 the rate has slowed to 1%. Brown attributes this to the exhaustion of the potential of the various techniques available to improve yield. From 1984 population has been growing faster than the grain supply.

Whatever further marginal improvements may be achieved in increasing the internal biological efficiency of plants to produce food, the ultimate limit is fixed by the rate of photosynthesis. Although some futuristic scenarios look to the possibility of increasing photosynthetic efficiency by genetic engineering there is ample evidence that sustainable limits are being reached or exceeded in many developing countries. In many areas where intensive farming is practiced environmental imperatives are leading to pressure to reduce efficiency raising inputs such as fertilizers. According to BROWN (1994), China has one of the smallest rates of population growth in the developing world adding currently 14 million people a year. By 2015 its grain import needs are likely to exceed the World's current exportable grain supplies. In Mexico yields have shown no increase in past nine years and there is an annual grain deficit of 5 million tons. Ethiopia is also deficitary and its population is expected to triple in the next four decades (Fig. 1). Any movement in these countries to increase the proportion of meat in the diet will further exacerbate the trend because of the low conversion factors for grain feed into meat yield.

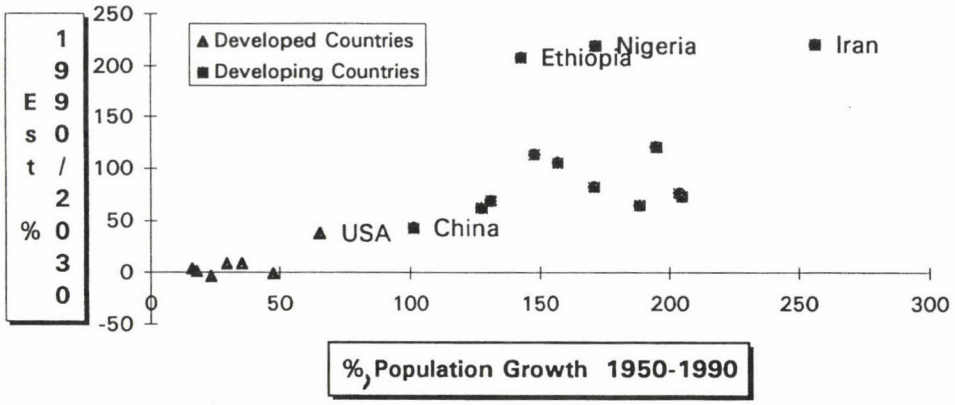

Fig. 1. Comparison of population growth for some most populous countries. Source BROWN (1994)

In addition, the current grain surpluses in the developed world are unlikely to be maintained for long. US grain exports dropped from 110 million tons in the early eighties to 70 million tons in the early nineties and, although population growth in the next four decades is not expected to exceed 1% per year the exportable surplus could very well disappear under the twin pressures of increasing demand for food and building land to meet population growth. For the World as a whole 1984 seems to have been a pivot point and the steady growth in grain production per head of population has turned, since that date, into a decline.

3. Water supply – a physical factor limiting production

In the last two decades of the twentieth century water supply has become a vital issue is Europe. In those countries where it was thought to be an inexhaustible resource, pricing strategies, that did not take into account a sustainable extraction level, have led to overconsumption and depletion of aquifers in Southern Europe. It has long been recognised that surface waters are not an inexhaustible sink for waste water and EU legislation has led the way in improving the water quality of both inland and coastal waters. Nevertheless a 'polluter pays' principle applied indiscriminately is a blunt instrument and more efficient protection closer to the source could be obtained by charging each source of effluent on the basis of the degree of restorative treatment needed.

Table 1

Water consumption for Mediterranean regions of some EU countries

Country	Total resource (10^9 m^3/yr)	Consumption (10^9 m^3/yr)				Consumption index (resource/ consumption)
		Potable	Industry	Agriculture	Total	
Spain	31.0	0.1	0.1	12.0	12.2	39.0
France	74.0	0.2	0.1	1.7	2.0	2.7
Greece	58.6	0.2	–	3.5	3.7	6.3
Italy	187.0	1.1	0.7	13.0	14.8	8.0

Source COLIN (1993)

According to COLIN (1990), the water supply crisis is being felt most severely in the Mediterranean area, particularly in Spain, as is shown in Table 1. He further indicates that the largest user of water in these countries is agriculture and its rapid development and industrialisation has led to a non-sustainable use of the water resource in some areas (Table 2). In the Mediterranean surface water persists in the summer months in rivers only where it is being supplied from large aquifers usually situated in remote mountainous areas. Local supplies are pumped from aquifers and they are often not replaced by winter rains. This "mining" of geological water reserves leads to two negative effects on food production. The recycling of water into and out of the aquifer with little replenishment from rain can lead to a build up of soluble salts in the soil with a strong negative effect on the agricultural value of the soil. This salinization phenomenon also occurs in other areas where the correct combination of rainfall and extraction occurs and the Hungarian Plain is one such area in Central Europe.

Table 2

Average water consumption per ha of irrigated land

Country	Millions of ha irrigated	% of total farmland	Consumption (billions of m^3 per year)	Unit consumption (m^3/ha/year)
Spain	3.3	12	20.0	6,000
France	1.2	4	3.6	3,000
Greece	1.2	12	3.5	2,700
Italy	3.6	21	13.0	3,600

Source COLIN (1993)

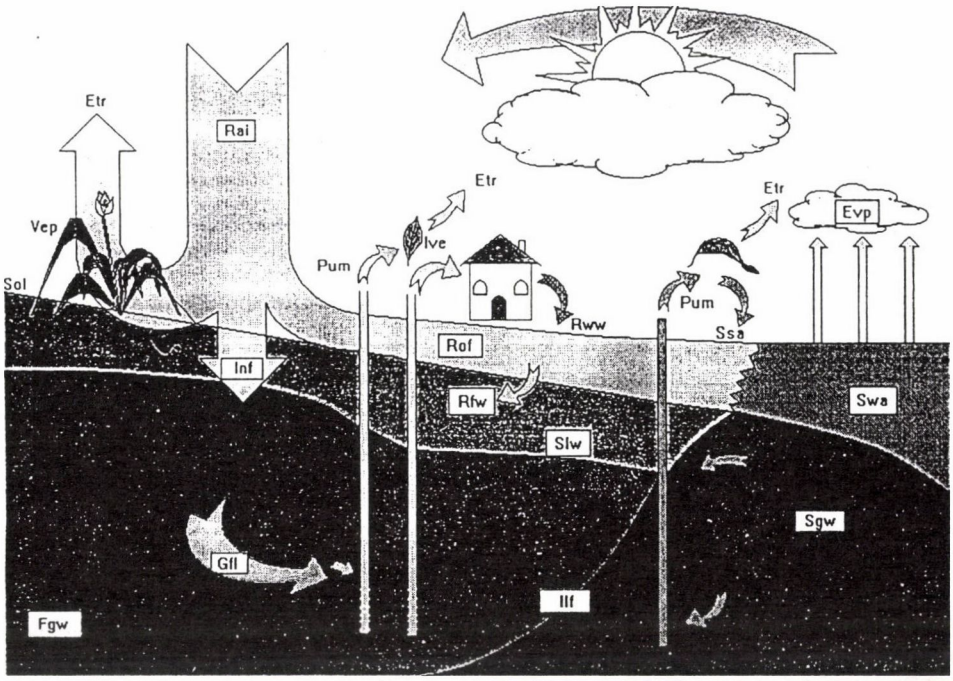

Fig. 2. Salinisation of a coastal aquifer by over extraction. Source PERGENT (1993)

In coastal areas extraction from the aquifer at a rate faster than replenishment by precipitation, seepage or underground inflow from higher areas leads to a reversal of the normal underground outflow from the land to the sea. As salt water is drawn into the aquifer the extracted well water becomes progressively saline and unusable for irrigation, human consumption or almost any other use. All of the Mediterranean coast of Spain is affected and COLIN (1990) shows that there is serious seawater intrusion into the coastal aquifers in Sardinia, Greece (Argolid plain), the Balearic islands and to a lesser extent in France (Fig. 2).

4. Climate change

Over the last decade a considerable debate has been raging over whether anthropogenic climate change is occurring at a significant rate or not. Inevitably some of those joining in the debate are influenced by pressure groups with vested interests in particular interpretations of the phenomena that are being observed. Whatever interpretation is placed on the data it is indisputable that concentrations of carbon dioxide and other greenhouse gases in the atmosphere are at historically high levels and are rising at an increasing rate.

A typical atmospheric carbon dioxide data set partly derived from direct measurements and partly from deconvoluted ice core data by SIEGENTHALER and OESCHGER (1987) is shown in Fig. 3. It can be seen that carbon dioxide levels have increased by 25% over the last two centuries about half the increase being over the last three decades. Data for carbon dioxide and other greenhouse gases measured at a large number of experimental sites throughout the world and collected by the US CENTER for GLOBAL ENVIRONMENTAL STUDIES (1993) are all showing an ineluctable rise. Palaeoclimate records derived from the Vostok ice cores by BARNOLA and co-workers (1987) (Fig. 4) show that there is a close correspondence between carbon dioxide levels in the Earth's atmosphere and mean temperature. Most scientists working in the field agree that the increase in greenhouse gases in the atmosphere from human activities is giving rise to global warming but that there is, as yet, insufficient evidence to predict the rate.

There is considerable concern about the possible consequences, all of which impact on agricultural production, so much so that 48% of the current budget for the European Union (EU) four year research programme on the environment (1994–1997) is devoted to global change. Global warming is expected to give a mean rise in temperature for Europe as a whole of 3 °C over 50 to 100 years. The impact of this warming is likely to be felt more through increased precipitation of up to 10% principally occurring in winter months and drier and hotter summers. The frequency and severity of extreme events such as storms and floods are also likely to increase.

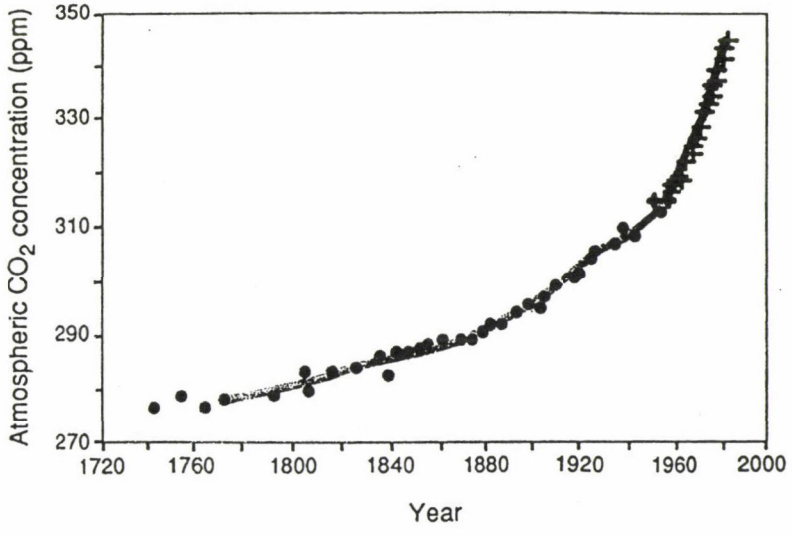

Fig. 3. Carbon dioxide levels in the atmosphere. Source SIEGENTHALER & OESCHGER (1986)

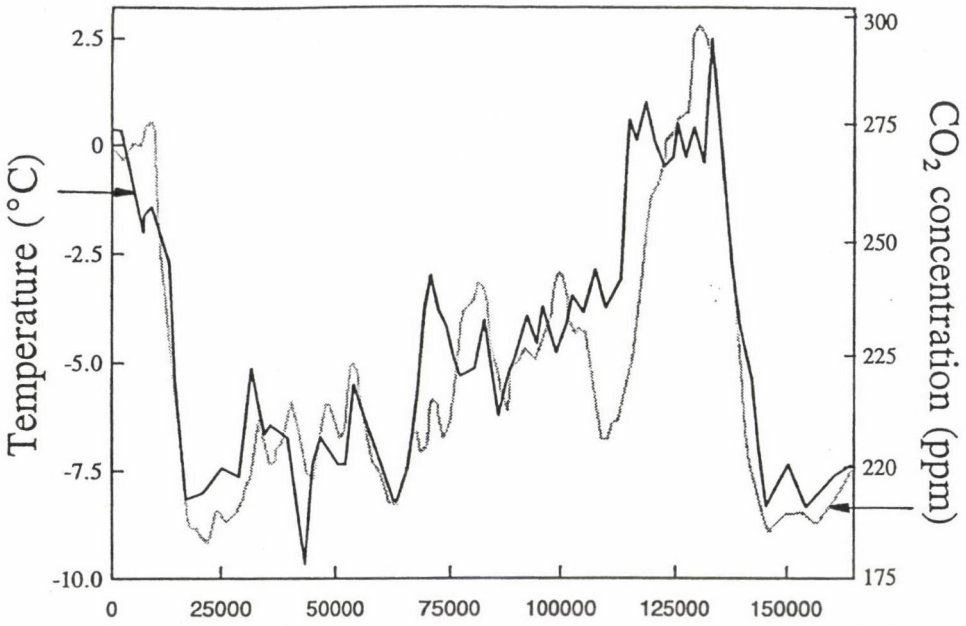

Fig. 4. Palaeoclimate temperature and carbon dioxide. Source BARNOLA and co-workers

Whatever the magnitude of these changes they will increase the climatic stresses on agricultural land already in many areas encountering production limitations. The Mediterranean area has already experienced a decrease in precipitation of 50% over the last 100 years and most experts predict that the future climate will be hotter and drier. This long term trend is being further exacerbated by a steady increase in population in the coastal zone and seasonal increases in tourist populations. On the southern shore of the Mediterranean which has a population growth of 6% per annum the situation is rapidly getting out of control and will be the major strategic international issue in this area of the next decade.

Climate change could also have some rather unexpected and subtle effects. PSENNER and SCHMIDT (1992) who studied two high mountain lakes in the Tyrol, hypothesised that the pH of sensitive lakes is affected not only by acid rain but also by temperature changes. The pH values calculated from diatom assemblages showed a strong correlation with mean temperature over 200 years (Fig. 5). Such changes in water chemistry brought about by climate change could have serious effects on agricultural productivity.

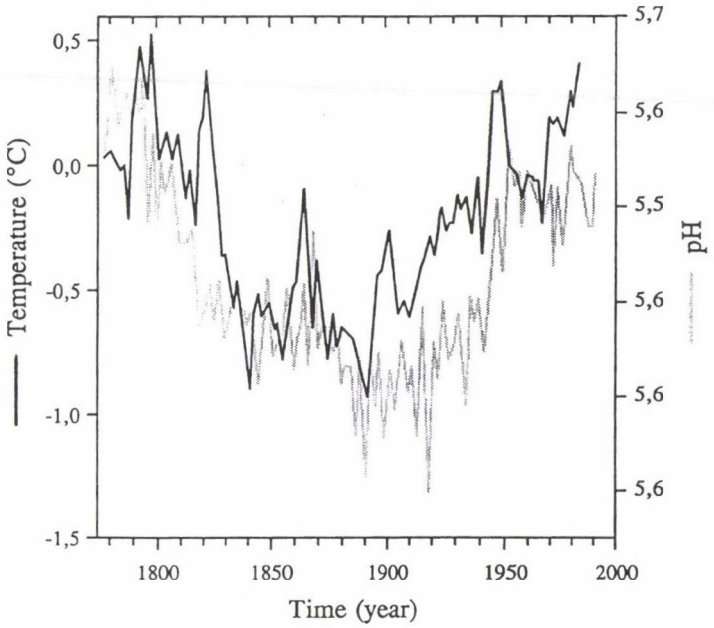

Fig. 5. pH and temperature of Alpine lake waters. Source PSENNER & SCHMIDT (1992)

It has been shown that the understanding of short term climate behaviour can have a beneficial effect as demonstrated by the use by Peru of El Niño Southern Oscillation (ENSO) based rainfall forecasts. According to the US GLOBAL RESEARCH PROGRAMME (1995) Peru was able to increase its agricultural production by 3% in 1987 despite a moderate ENSO occurrence in 1986–1987 compared with a fall of 14% in 1983 following the severe ENSO event in 1982–1983. However the first effects of global warming are expected to be related to a greater pick up of humidity from the world's oceans which will result in increased precipitation and a greater likelihood of extreme events. The increased energy being handled by the atmosphere will lead to greater instability, a higher frequency of extreme events and a reduction of predictability. All these trends will make successful food production more difficult.

The pollution of food by environmental contaminants

5.1. Airborne pathways

The mobility of contaminants in the atmosphere, the dynamics of deposition and the importance of food as a transport mechanism for these contaminants was demonstrated in a dramatic way in 1986 by the Chernobyl reactor incident.

Although generally weather systems in Europe move from west to east during various phases of the release contaminated air deposited significant levels of radioactivity on growing crops up to 2000 km west of the source. The fallout from the atmosphere was determined to a large extent by precipitation rather than distance from the site and rates of deposition differing by factors up to 1000 were observed between sites a few hundred metres apart where local thunder showers had occurred. The recycling of caesium through the soil/animal fodder nutrient chain was another rather unexpected phenomena brought to light by Chernobyl.

GRAY (1991) describes the severe effect on trade in agricultural products since food was the major vector for exposure outside the immediate vicinity of the reactor and between 60% and 80% of the total radiation dose to the population came from foodstuffs. Pathways into man were principally through animal products between 30% and 60% of the food borne dose arising from milk. Although measures were taken by authorities to limit the contamination of food offered for sale their effect on the overall dose was probably very small as they only resulted in the removal of a very few contaminated lots from the food chain.

The European River and Ocean System (EROS) project of the EU Environment Research Programme has given some interesting insights into pollution mobility. The EROS project grouped a number of specific research projects investigating the pathways through which contaminants moved through the river systems into the Western Mediterranean.

One of the most unexpected facts to emerge is the relative importance of airborne pathways in transferring contaminants into the ocean. While over 75% of phosphorus and over 60% of nitrogen transferred into the Western Mediterranean are river-borne, more than 70% of dissolved copper, 80% of particulate copper and cadmium and more than 90% of particulate and dissolved lead are airborne.

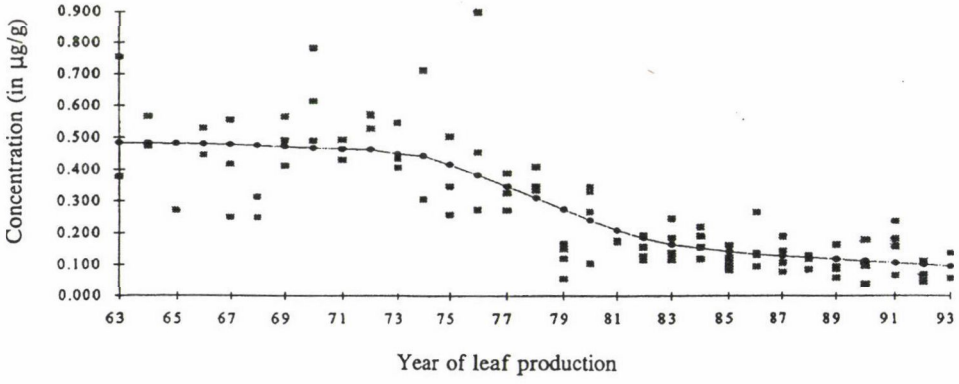

Fig. 6. Mercury in the Mediterranean derived from plant scales. Levels and effects of pollution. Evolution of mercury found in fish scales in Marseille. Source PERGENT (1993)

Environmental legislation is frequently criticised by some European industrialists as harming their competitivity by imposing an unnecessary burden on their operations. Nevertheless The NATIONAL FOOD AGENCY of DENMARK (1990) reporting on contaminants in food states that the average lead intake in food for a child of 10 kg fell from 100 μg per week in 1980 to 45 μg per week in 1987. This decrease is attributed to the reduction of lead in petrol brought about by EU legislation. The Provisional Tolerable Daily Intake of lead from all sources established by the WHO/FAO expert group in 1986 gives a recommended maximum intake from all sources for a 10 kg child at 250 μg per week showing that the reduction effected is clearly an important contribution to public health.

Within the framework of the EROS project work has been carried out by the University of Corsica on the change in mercury contamination occurring in different parts of the Mediterranean. PERGENT (1993) has derived historical mercury levels by analysing the mercury contained in the scales of a marine plant which form serially along the root during each season of growth (Fig. 6). These measurements show that an overall decrease of about a factor of 5 occurred between 1963 and 1993 with 60% of the decrease occurring between 1973 and 1983.

Comparative measurements of mercury in seawater in the Mediterranean (Table 3) show a higher concentration in the Gulf of Lions i.e. off the mouth of the Rhone than in the Alboran Sea i.e. near the Straits of Gibraltar where there is inflowing water from the Atlantic. Levels in the Atlantic are lower but close to those in the Alboran Sea and levels in the Pacific still lower. These are reflected in the levels of mercury in Tuna taken from the respective waters.

Table 3

Dissolved mercury in seawater

European river and ocean systems	
Total dissolved mercury in seawater pM[a]	
Gulf of Lions	1–7
Alboran Sea	1–4
Atlantic (Fitzgerald et al.)	1–3
Pacific (Gill and Bruland)	0.5–2.5

[a]1pM = 2 ng l^{-1}

Source EROS Brochure, European Commission

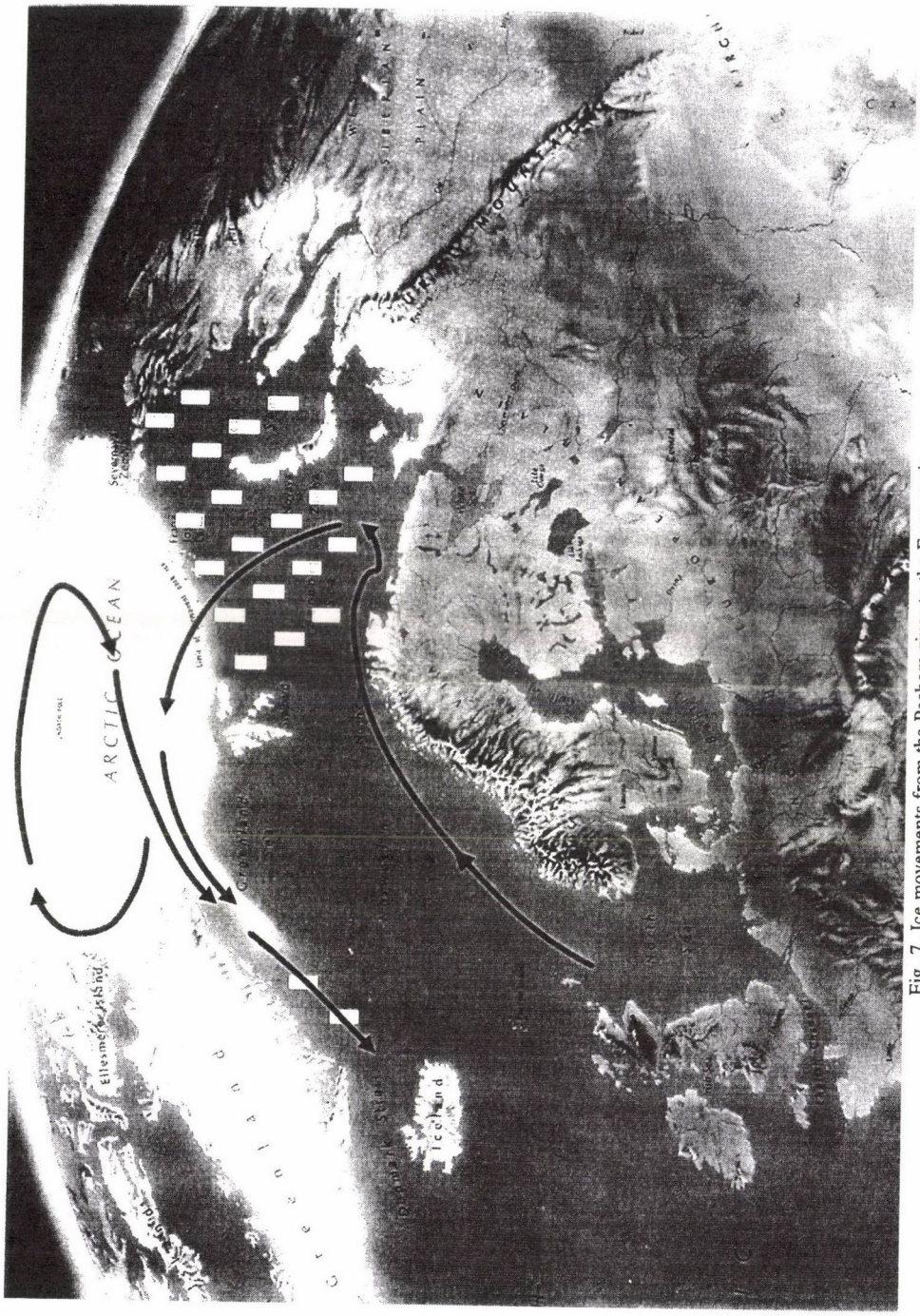

Fig. 7. Ice movements from the Pechora river to the Fram strait

5.2. River sediment, sea ice, marine pollution cycles

The Arctic seems as unlikely place from which a threat to our food supply could originate. STANNERS and BOURDEAU (1995) report, in the Dobris assessment on the State of Europe's Environment, that the seas bordering the European Arctic are among the least polluted in Europe. However the special topography of the Arctic Ocean makes it not only one of the major motors of climate but also of unique biogeochemical transfer systems which could be a major contamination sources for the European food chain.

The global ocean thermohaline circulation is described by SCHMITZ (1995). Formation of Arctic ice in winter generates cold saline water which descends to the depths of the ocean and eventually leaves by the only deep water channel available to it between Iceland and Norway. This deep water current traverses the Atlantic from North to South picking up more cold water from the Antarctic Ice shelf where it turns eastward across the Indian Ocean to surface in the Pacific. The counterflow surface current retraces this journey passing eventually around the West Indies to become the Gulf Stream, a major moderator of West European climate.

An unexpected effect of global warming could be that a reduction in sea ice formation in the north polar regions could reduce or stop this conveyor belt and hence the Gulf Stream, resulting in a fall in temperature of Western Europe with all the consequences this could have for agriculture.

Much polar ice forms in the winter in the shallow shelf seas which fringe the Arctic ocean depths and in the winter ice some ice is formed at the bottom of these seas occluding and transporting sediment to the surface. An important source of such ice borne sediment is the Pechora river and most of the sediment deposited at the mouth in the May melt of the river is picked up by the ice formation in the following winter and moves with the pack ice towards the Fram Strait between Greenland and Svalbard (Spitzbergen), collecting during its transit airborne pollutants on the ice surface (Fig. 7). The Spring melt of the ice floes releases the accumulated pollutants at the ice shelf edge into one of the most important areas for phytoplankton formation which is stimulated into a spring bloom by nutrients which have also been transported with the ice as described by PFIRMAN and co-workers (1995).

The phytoplankton is consumed by krill that is in turn consumed by capelin, which are together with whitebait a member of the smelt family. These fish are characterised by a fatty adipose fin near the tail and they are a food source for larger species of fish, marine mammals and birds. During this food transfer process there is a transfer and concentration of lipids which can reach levels as high as 50% at the most

favourable season (Fig. 8). The lipids transferred along the chain will occlude any chlorinated hydrocarbons in the river sediments thus forming a direct food contaminant source in a sea area which is a nursery for many North Atlantic fish.

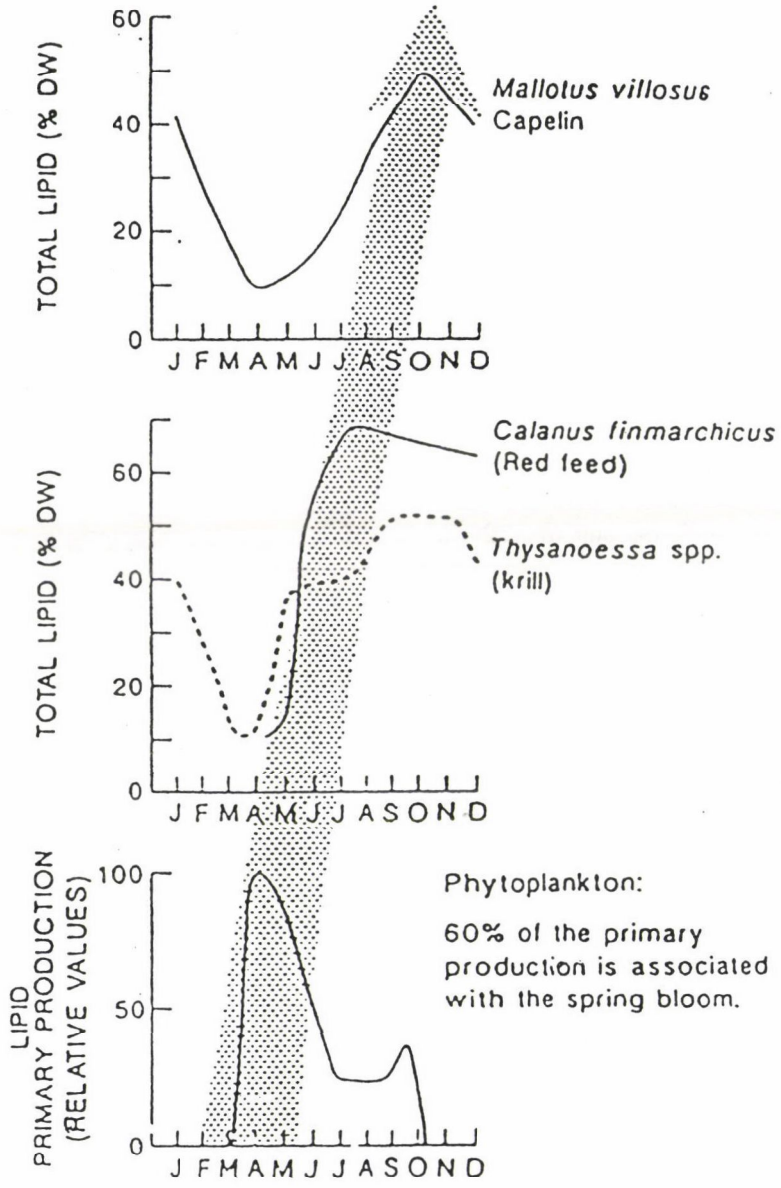

Fig. 8. Lipid chain from phytoplankton to fish

The Pechora River is 1 802 km long and flows through the Northeastern part of European Russia from the Ural Mountains to the Barents Sea. Its drainage basin, West of the Urals, is about 326 000 sq km, that is as large as the surface area of France, and has extensive coalfields. To the East of the Pechora river the Yamal peninsula contains more oil reserves than the Arabian peninsula. The current need to revive the economies of the countries of the former USSR will create pressures for a rapid and uncontrolled exploitation of resources around the Eurasian Arctic. The fragile ecology and the unique biogeochemical transport systems of the region may very well produce some further unpleasant surprises as sources of food contamination.

At a conference of the Society for Environmental Toxicology and Chemistry (SETAC) in Copenhagen in June 1995 a special session was devoted to discuss the concern that some chlorinated hydrocarbons may be acting as hormones stimulating a sexual change in fish. This was based on research carried out in the North Sea where it had been cited as a possible explanation for the preponderance of males and a consequent fall off in replacement rate of certain flatfish species observed in the Elbe estuary. If confirmed, this phenomenon, could also be contributing to a reduction of fish production in North Atlantic waters by pollution deriving from the ice transport mechanism.

5.3. Need for pollution control at source

The few cases dealt with above illustrate that, rather than remove contaminants from human food, it is much more effective to control them as close to the source as possible. This approach has a number of advantages:

- It reduces the overall cost because the amount of material to be processed is less than if the contaminant is dispersed;
- It removes uncertainties regarding bioaccumulation in the human food chain;
- It reduces the risk of other damage to the ecosystem.

A typical example is the case of cadmium pollution which is extensively examined in the report of the ENQUETE COMMISSION of the GERMAN BUNDESTAG on the PROTECTION of HUMANITY and the ENVIRONMENT (1994). In non smokers 96% of cadmium enters the human body through food and 4% through drinking water. In non smokers cadmium levels reach 10–40% of the provisional Tolerable Daily Intake recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). This is already a level which calls for action to reduce the body burden especially since tobacco consumption can double this figure. The Committee concludes that "Effective Substance Chain Management" i.e. control of all inputs to the environment is the only way of achieving this reduction.

Effective Substance Chain Management uses a variety of assessment criteria to evaluate material flow and its effects. These are:

- *exposure*
  environmental compartments
  population
  workplaces
- *behaviour in the environment*
  biotic and abiotic degradability
  susceptibility to mobilisation
  susceptibility to accumulation
  bioavailability
- *human toxicity*
  general toxic effects
  behaviour in the human organism
  carcinogenicity
  risk/unpredictable risk
- *ecotoxicity*
  terrestrial systems
  hydrosphere
  combined effects
  risk/unpredictable risk.

Cadmium is a by-product of zinc smelting so that its production depends directly on zinc production and if cadmium use is reduced below the byproduct production rate level effective measures have to be taken to deal with the unused metal. Under 'effective substance chain management' cadmium inputs are reduced as far as possible and sinks, which make ecological and economic sense, are utilised to ensure a permanent fixation of the metal.

The committee reports a 50% drop in airborne particulate deposition of cadmium between 1981 and 1982 so that, as with airborne lead, the control of emissions is proving to be effective. The report also recommends the use of low cadmium phosphate ores for the production of fertilizers.

6. The challenge

The vital question is, can we ensure an adequate and wholesome supply of food for the world's population in the twenty first century? CHEN and KATES (1994) estimate that in 1990 15–35 million people were at risk from famine, 786 million were

vulnerable to chronic under-nutrition and hundreds of millions suffered from micronutrient deficiencies and disease. They however proffer a 'normative scenario' achieved by a fundamental shift in the political system in which food security is achieved in a warmer, more crowded, more corrected but more diverse world in 2060. They themselves assess their vision as 'speculative and clearly optimistic'. The essential prerequisite for this scenario is a demographic transition from a developing world with high births and high deaths to a world with low births and low deaths. The development of sustainable society, including a sustainable agriculture, on a world and a regional basis is also necessary and from the few examples quoted in this paper it is clear that current systems are already 'creaking at the seams'.

Our economic philosophy is rooted in a system of values developed in an infant industrial, colonial society that assumed that wealth increased with the growth of economic activity. The fact that an increasing proportion of wealth in developed society is being devoted to the rectification of environmental damage caused by other 'wealth' creating activities indicates that the present market system will not lead to world economy to sustainability. A market economy in which the system of values used takes into account the economic impact of the depletion and degradation of natural resources could very well do so. Economists are attempting to develop such a system which is described by VAN DIEREN (1995) in the report to the Club of Rome 'Taking Nature into Account'.

The few examples given in this paper show that the environment is complex and full of unexpected phenomena. The need for a continued coordinated research effort and continued vigilance on the part of environmental scientists has never been greater. The EU programme is enhancing the value of national research by its coordinated approach to such vital questions as stratospheric ozone, terristrial ecosystems (TERI) and coastal ecosystems (ELOISE). In contrast with other areas of research the planet is the laboratory in which environmental research is made. The collection of coherent data sets by comparable methods in different geographical areas allows environmental systems to be studied 'in extenso' the value of this approach being clearly demonstrated in the two European Northern hemisphere ozone experiments in which over 100 laboratories participated. The EU research programme also focusses on European contributions to global programmes such as the International Geosphere Biosphere Programme.

Agricultural and food science also has a vital part to play towards sustainability in increasing yield and efficiency, conserving the harvest and improving the efficiency of food production and conversion. Whatever their discipline, all scientists must be able to communicate in such a way as to turn their knowledge of natural science into understanding by the population at large and particularly by policy makers. During the

latter half of this century society has been bewitched by technology so that many believe that it will provide a solution to all problems. The true goal of science is to understand and not to conquer nature, for after all it is the natural world that provides humanity with that fundamental essential of life – food.

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The views expressed in this paper are those of the author and do not necessarily represent those of his institution.

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FOOD SCIENCE AND INDUSTRY

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Throughout the 20th century, the food and beverage processing industry has provided an ever increasing array of products which have never been as tasty, nutritious and safe as in the past. Food Science is multidisciplinary and its applications span from the farm to the fork. One can recognise immediately its complexity and its importance if one considers that food is the world's most intricate product and that any individual absorbs close to one hundred ton of food and drink over a life span.

In this short paper, I would like to offer a few thoughts on the relationship between Food Science and the Industry from the point of view of both the scientist and the industrialist.

1. Trends

The success of research in Food Science will depend on recognising the emergence of a number of trends:

1.1. Scientific research

From a general perspective, one can delimit three logic's underlying scientific research: the political, the industrial and the financial logic. Today the financial logic seems to prevail almost everywhere. Food companies follow a similar path. Many of them have undergone major reorganisations to streamline their activities. Scientific and technological research is closer to business than ever. It is not an issue of short term versus long term research. The key is to make sure that R & D plays its proper role in the corporation and protects its specific know-how.

1.2. Consumer trends

The 20th century has brought an increasing self-expression from a biological, behavioural and social point of view. The sense of the individual has overcome that of the group. In terms of food, this is reflected by the fact that individuals are more selective in their food choice. As a consequence, the dominant forces driving demand in the food market today are:

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- Greater variety
- Greater convenience
- Higher quality

How people select their own food and what are the determinants for food selection are still questions poorly understood by psychologists and nutritionists. This is clearly an area which deserves more attention.

1.3. Socio-demographic changes

The world population will increase (mainly in developing countries) from 4.8 thousand million in 1985 to 6.1 thousand million in 2000 and may reach 8.1 thousand million in 2025. The fastest growing segment of the population is the elderly, in both industrialised and developing countries (e.g. in China the population above 65 years of age will nearly quadruple within the next 50 years to reach 270 million people). In Europe the age group over 60 is increasing to reach 26.7% of the population in 2020 (15.5% in 1965), while the age group 1–19 will represent 20.4% of the population (31.8% in 1965). Before the middle of the next century, developed nations may have more grandparents than children.

1.4. Food laws

Following the GATT Agreement, it is obvious that free trade implies harmonisation and agreement on standards. International harmonisation of food laws (i.e. on packaging, approval of ingredients, biotechnology, irradiation, etc.) will bring more products to the consumer and open new markets.

2. Collaborative research

2.1. Collaboration between industry and universities

The concentration in the food industry tends to result in more focused R & D functions. In turn, this provides greater product innovation and proliferation of products. Although large food companies have their own R & D facilities, it will be increasingly difficult for them to keep up with the fast development of science. Moreover, as the industry requires faster results from its scientists, this leads to a potential conflict between flexibility and expertise. It is difficult to become an expert on moving targets. To keep up with the pace of food science and related disciplines, the food industry will have to further improve synergy with academic research in key area. Hence the necessity for academic researchers to better understand the constraints of the food industry with respect to production cost, legislation, consumer attitudes,

etc. It is also imperative that they appreciate who should own intellectual property. On his side, the entrepreneur who finances research in the public sector should understand that the right to publish is a key element in maintaining a high level in academic research.

2.2. Collaboration between academia, government and industry

The food industry has realised that health and well-being, the quality of food, and the measure to take to protect the environment require the collaborative efforts of many experts and organisations, both private and public. The International Life Sciences Institute (ILSI) is one of the major organisations that meets this end.

ILSI is an international non-profit public foundation, established in 1978, to advance the sciences of nutrition, toxicity, risk assessment and environmental safety. This organisation provides a neutral ground where academia, government and the industry can unite their effort to seek scientific solutions to such important public issues as:

- the role of nutrition in human health
- the safety of food ingredients and additives
- world-wide malnutrition
- food-related allergies
- safe water and air
- chemical safety and environmental health
- identification, assessment, and management of human health risks; and universal global standards for articulating and interpreting pathology and toxicology test results.

ILSI has branches in many part of the world, including Europe, where programmes are developed in response to scientific issues of regional significance.

3. Education

Up to the end of the 18th century, scientists were researching above all to understand natural phenomenons. Today, scientists conduct research in order to act. Scholars have become researchers. There is a growing inventory of scientific knowledge needed for food science and nutrition. Research will keep pace only if young food scientists are trained to assimilate and utilise advanced knowledge while keeping their feet on the ground. However Food Science is not rated as glamorous research by many students. One should maybe remind them that in classical times

professional cooks in Greece were the most respected of all artisans and the most highly paid!

Both the private and the public sector need researchers of high calibre. "Quality is never an accident; it is always the result of intelligent effort" (John Ruskin). Hence the necessity of meeting high standards in teaching Food Science. In Europe, there is no lack of possibilities to obtain a first rate training in Food Science and related disciplines. When academic programmes are tuned to the need of the private sector, students can almost be guaranteed to find a job.

Different programmes have been recently established in Europe to further educate students interested in food sciences. For instance, the European Masters in Food Studies, an 18-month postgraduate programme, was initiated by leading European Universities, with the active support of the European Commission to provide scientific and business training in an international context, and to create awareness of the cultural, social and economic realities of the modern European food industry.

Food scientists must work hand in hand with nutritionists since consumers are increasingly becoming aware of the relationship between food and health (they still have objectives which may not satisfy nutritionists!). Students in Food Science should be made aware of the European Nutrition Leadership Programme organised by Prof. J. Hautvast with leading European nutritionists in collaboration with EU, food industry and European nutrition organisations and societies. The objective of this programme is to organise regularly advanced training seminars for Ph. D students and postdoctoral fellows with an emphasis on nutrition science, future strategies and new frontiers in nutrition research.

4. Products and processes

One of the characteristics of the food industry is its slow evolution towards technological changes. The true innovations which will appear on the food market 10 years from now are based on technologies and scientific concepts known today. Among many of them, biotechnology and process modelling have great potential.

4.1. New technologies

There is no doubt that the food industry will make sooner or later a large use of "new" biotechnology in food processing. It will help not only at modifying the texture or taste of food products, but also at improving their nutritional quality and safety. Raw materials, ingredients and processes are all amenable to considerable improvement through biotechnology.

The consumer distrusts new technologies, largely on the basis of fear, misinformation and misconception. Biotechnology is especially under pressure. On the one hand the consumer does not understand the risks involved and on the other hand he does not believe that the industry is able to control new technologies. The public opposes, the legislator reacts with restrictions on new products and processes and may take measures which ultimately will impede scientific progress and restrict benefit to the humanity. As Thomas Jefferson (1743–1826) already noted: "The best government regulates only to prevent people from hurting each other, but not enough to impede the progress".

However traditional biotechnologies are far from having exhausted their possibilities. What is old is not necessarily outdated. Researchers have to rethink with a fresh look, modify and industrialise traditional methods which were empirically developed in order to find new products and to valorise new raw materials.

4.2. Process modelling

The food industry is far behind the chemical industry in modelling. Modelling cannot be done by amateurs. Food production managers are still shy of mathematical modelling. Models can indicate that experimental data are not as good as they believe to be or can also bring results contrary to their experience. Modelling will contribute at developing new technologies or newly adapted technologies, but also at improving existing processes through process control and automation.

5. Technology transfer

Competitive advantage can only be maintained by new ideas. In term of investment, the challenge of transforming research into competitive advantage is enormous. A bad choice and a bad strategy can cause important damages.

New ideas are generally costly. Profitability is not immediate and many of them cannot be used. It is therefore tempting to rely increasingly on technology transfer. Technology transfer is a process by which technological innovations developed in one institution are discovered, acquired, and adapted for use by another institution. For example, HACCP analysis was developed by a food company under contract with NASA. Irradiation technology and magnetons for microwaves ovens came from the defence industry.

Technology transfer brings innovation (often by cross-fertilisation), shortens development cycle, reduces time to the market. Dramatic changes in technology transfer have come through information technology. More than 11'900 industrial R & D organisations in the USA are listed in data bases. Non profit R & D Centres

amounts to 27'000 organisations world-wide. This wealth of data and ideas is far from being fully exploited. Hence the pivotal role of library and librarians in helping researchers to identify information relevant to their work.

The academic world is also following the same evolution. In the past, its role, albeit highly important, was limited to the transfer of knowledge, through the hiring of educated students. Today, the University participate more and more to the transfer of true technology.

6. Challenges

There is no lack of challenges today for food scientists. Among many, I would like to mention only four of them.

6.1. Speedier response to the market

Changes in lifestyle, particularly in developed countries, are more rapid than ever. Today one can only lean on what moves. The number of new food products is ever increasing (with a concomitant rate of failure). In the USA for instance, 15'006 new food products were introduced on the market in 1994 (9192 in 1989). It is granted that these new products are so-called new storage keeping units, i.e. a new product introduced into two package sizes is counted as two. The number of actual new products is probably more in the range of 5 000–6 000 which is still a very high figure. This means that research (both academic and industrial) must be carefully programmed to deliver results on time.

6.2. New technologies

Researchers must constantly be on the alert. New raw materials, new packaging, information technology, biotechnology, sensor technology are already shaping the industry of the future.

6.3. Multi-Hurdle Technology

Multi-Hurdle Technology is an intelligent combination of several food preservation methods to produce minimally processed foods. It is a challenge to food producers to find new ways to prevent raw materials from contamination by microbial pathogens and food from spoilage by micro-organisms. Mastering and combining hurdles will provide new solutions.

6.4. Control of water activity

Products quality and shelf-life (i.e. control of water activity) will be further improved through a better understanding of the peculiar properties of water.

Chemically, nothing is more simple than a molecule of water; yet, liquid water, the most researched fluid of all time, continues to baffle scientists seeking its molecular structure. So familiar is water to us, that we forget that water has not only unique chemical and physical properties, but also exceptional biological properties as a nutrient: it is the only liquid which can sustain life.

7. Conclusion

Tremendous challenges must be met soon by the food industry, especially in developing countries. This will require the skill, knowledge, energy and imagination of well trained and open-minded young scientists. As William Phelps said: "At a certain age, some people's mind close up. They live on their intellectual fat."

"The creative act and the innovative process may be regarded as the fuel and the motor of the industrial enterprise" (Albert Westwood). New technologies will probably have a greater influence on the skill requirements of food scientists than other factors in the future. However food scientists are only one link in a long process during which a number of specialists intervene. They must work hand in hand with their business counterparts, while keeping themselves alert on the front advancement of many disciplines, including nutrition. "It is pardonable to be defeated but never to be surprised" (Frederick the Great).

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SUSTAINABLE DEVELOPMENT OF HUNGARIAN AGRICULTURE

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The term 'sustainable development' was not yet mentioned at the first world conference on environmental protection, which was held in Stockholm in 1972 under the title: UN Conference on Human Environment. However, at the second similar world-wide meeting held twenty years later in Rio de Janeiro, this was already the most fashionable and most frequently cited term. The Rio Conference set it as a global political objective to realize sustainable development everywhere and in every field, i.e. in every country, whether advanced or developing, and in each branch of the economy, ranging from settlement policy to agriculture and the food industry. Sustainable development was voiced by both the representatives of governments and the activist of social movements. Practically this was the only issue on which they had an agreement. This might be suspicious in itself, because it might imply that neither of the parties concerned really see the main point underlying this term, but it may also mean that both parties conceal their own particular ideas behind this broad term which is flexible enough to embrace almost everything.

Following the Rio Conference, the concept of sustainable development has undergone a certain devaluation. It had been used so many times without any substantial content that it was not taken seriously even when its use was professionally or scientifically well-founded and justifiable.

I shall try here to give a brief historical outline of the concept of sustainable development. There was a period between 1984 and 1987, when as a member of the World Commission on Environment and Development, led by Norwegian Prime Minister Ms Brundtland, I had an opportunity to take a close look at the development of this concept, as it finally appeared in the report of the Commission. So I also have some personal experiences in this field. Placed in a historical frame, there is a better opportunity to give a deeper insight and evaluation of the real situation.

The term sustainable development began to spread in the literature in the early 1980s. Lester R. Brown, President of the Worldwatch Institute, published his book entitled Building a Sustainable Society in 1981. In this book, the author maps out the

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course to be followed to reach sustainability, including such things as the stability of population, the conservation of natural resources, the utilization of renewable energies and the protection of the natural values. Changing over to sustainability also requires the transformation of institutions, the economy and the system of values.

The World Commission on Environment and Development published its report entitled Our Common Future in 1987. As its main message, this report encouraged the global use and application of the concept of sustainable development. The report presented a development model which included the elements of both quantitative and qualitative development, pointing out, however, that the internal proportions of these would necessarily vary from one country to another, according to the actual needs. In case of the developing countries, though quantitative growth will be predominant in the coming period, qualitative development will also be required. In case of the industrially advanced countries, further quantitative growth is generally not considered as the primary goal. Instead, qualitative changes are emphasized in both production and consumption. The introduction of environment-friendly technologies and the saving of resources are, of course, essential requirements in both cases.

The report Our Common Future defines the concept of sustainable development in a concise manner:

"Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs."

This formulation reflects essentially a political stand. It concentrates on Man whose primary needs must be satisfied, while those of future generations should also be taken into account. The protection of the environment and resource saving are not specifically mentioned in this definition. Thus the concept of sustainable development is a message for the world's developing countries, as well as for the low-income strata of society, to give them some hope as to their future. An indirect interpretation of the definition, however, makes it clear that future generations may only share in the worldly goods if natural resources will be saved on a wide scale and if the values of the environment will be preserved.

It was in the mid-1980s that the elaboration and definition of the criteria of sustainability for the individual sectors of the economy started. In the opinion of many experts, the sustainable development of any economic sector can only be attained if both social and economic policy will be formulated in compliance with the definition of sustainable development. Otherwise, the sustainable development of any sector, e.g. industry or transportation is doomed to failure.

As regards agriculture, we should first of all answer the question whether bio-farming is the same as sustainable agriculture. My definite answer to this question is that bio-farming is only one of the several forms in which sustainable agriculture may

appear. On the one hand, it must be underlined that sustainable agricultural production does not prohibit the rational and controlled use of chemical fertilizers and pesticides. On the other hand, bio-farming, if used as a production system on a large area, would not be able to satisfy the basic needs for foodstuff. If there were bio-farming on the whole agricultural area of Hungary, for example, immense social problems would arise on account of defective food supply. At the same time, the bio-farming methods deserve support in the so-called hobby-gardens where no professional guarantees exist for the sound use of chemical fertilizers and pesticides.

The Food and Agricultural Organization of the United Nations (FAO) adopted the following definition of sustainable agricultural development in 1988.

"Sustainable development is the management and conservation of the natural resource base, and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations. Such sustainable development (in agriculture, forestry and fisheries sectors) conserves land, water, plant and animal genetic resources, is environmentally non-degrading, technically appropriate, economically viable, and socially acceptable."

Of course, there are definitions shorter than this, but the one formulated by FAO should be known, as it is the most competent inter-governmental organization.

The definition formulated jointly by IUCN, UNEP and WWF tends to emphasize the biological elements:

"Sustainable development is improving the quality of human life while living within the carrying capacity of supporting ecosystems."

According to M. S. Swaminathan's definition:

"Sustainable nutrition security is providing physical and economic access to balanced diets and safe drinking water to people at all times."

Invited by the Ministry of Agriculture a group of experts had completed their task last month in Hungary. They have elaborated a conception of sustainable agriculture best suited to the country's natural endowments. This working group consisted of leading scientists and experts of agricultural research institutes and universities. The draft of this conception had been discussed at six regional meetings. As a result, the following main principles for the sustainable development of Hungarian agriculture had crystallized:

It should

- be environmentally non-degrading,
- be resource saving,
- produce healthy food and fodder,

- make the present and future generations of farmers interested in agricultural production.

It has to be added to these general principles that sustainability should also be asserted in a regional context as well. In other words, regional development should be shaped so that production and the use of resources could be harmonized not only in the details, but also in its entirety.

As a farming system, sustainable agricultural development essentially endeavours to break with the agricultural system having been so popular in Hungary in the 1970s, which worked with very high energy and material input. The new system aims to employ environmentally sound, energy- and material-saving processes, placing special emphasis on quality. This new farming system can only achieve good results with adequate expertise. So research, education, innovation and extension services are to play a prominent role.

In working out the conception of sustainable agriculture in Hungary certain strategic goals must also be taken into account. In this respect three types of strategy can be conceived:

- no attempt is made at self-sufficiency in food: instead, there is considerable import,
- self-sufficiency is the basic goal to be attained:
- over and above self-sufficiency, there is a considerable amount of food export.

Before chosing any of these strategies, it has to be taken into account that Hungary is seriously indebted abroad: its gross debt stock amounts to 30 billion USD. That is, currently some one third of the state budget is spent on the repayment of interests and short-term bank credits. The country is unable to get rid of this burden in the foreseeable future. Under such circumstances, it would be a mistake to miss any opportunity which promises income for the Hungarian economy.

Another factor to be considered in decision-making is that the country's natural endowments – i.e. the size and quality of its agricultural land, the water resources and climate – make it possible to produce a significant amount of bio-mass of varied composition. According to theoretical calculations, Hungary with its population of 10.5 million could supply 18 to 19 million people with foodstuff. Its natural and biological resources would make such production possible.

With all this considered, of the three possible strategies, only the third one, namely self-sufficiency with a considerable amount of food export, seems to be feasible. However, this opinion is not shared by every politician and economist. In fact, there are many experts who propose a marked reduction in agricultural production in an effort to facilitate the country's joining the European Union. In my opinion – and

this is held by many other experts, too – the principle of self-sufficiency in basic foodstuffs should be asserted. Otherwise, there will be a further rise in unemployment, and not only in the rural areas, but also in some branches of industry for which agriculture means the domestic market. Furthermore, importing large quantities of foodstuffs requires financial resources. It is to be feared that such a step would lead to the further impoverishment of certain strata of society.

The principle of self-sufficiency does not exclude the import of foodstuffs. Import is essential to increase the competitiveness of domestic products and also the choice of goods.

As regards exports, marked structural changes are needed Hungary's accession to the European Union seems to be a historical necessity. Otherwise Hungary would be pushed to the periphery of Europe. The country's new export structure has to be developed in accordance with the agricultural policy of the European Union, and from this should those production-related tasks be derived which, in turn, should be realized in compliance with the principles and criteria of sustainable development.

I would like to underline two factors:

- It can be taken for sure that there will be an increase in the activities in agriculture and forestry not related to food production. This will also be part of the structural change.
- In the field of exports, the main emphasis will be laid on value and quality, rather than on quantity. Thus processing will certainly play an eminent role. Therefore, it is the primary interest of Hungary to develop an up-to-date food industry which is capable of producing competitive goods.

Sustainable development will lead to a higher regional differentiation in Hungarian agricultural production. While intensive plant cultivation will develop in regions of more favourable natural endowments, production will be extensive in the less favourable ones, where part of the available arable land will be changed into pastures or will be afforested, including the possibility of energy plantation too. Some kind of cultivation or grazing in necessary also in such regions where field is poor. Otherwise it is impossible to retain part of the local population and to prevent them from increasing the number of poor urban social groups.

Sustainable agricultural production requires the better utilization of the internal natural and biological reserves as well. In the coming period, therefore, those possibilities will gain in importance, which promote the better utilization of the existing natural and biological endowments and the internal genetic and physiological properties. There is a wide range of possibilities in this field. Belonging among them is, first of all, the protection of the arable land from various degradation processes and

effects. A production structure adjusted to the landscape or to the micro-region also means the exploration of the available natural reserves.

For the time being, the state still provides subsidies for agricultural production. However, this state support is markedly less than it used to be before the country's political change-over, i.e. in the period of the centrally planned economy. The present system of state subsidy also requires differentiation, because different economic incentives are needed, for example, in the case of a consciously undertaken extensive production system.

With plants and livestock, such existing properties or ones which can be achieved through improvement are to come into prominence as, for instance, natural resistance, stress tolerance, better utilization of the genetic potential or the improvement of quality indices. The biological fixation of nitrogen will also increase in importance.

The domestically produced bio-mass should be fully utilized. By a more rational utilization of grasslands and pastures and by means of some by-products could be significantly raised. Increasing the non-food-related production of the bio-mass would permit the improvement of the agricultural sector's energy balance in such a manner as to decrease the importance of the external sources of energy.

The application of re-cycling, wherever possible, will also come into the highlight. The utilization of by-products of the food industry in animal husbandry has long traditions in Hungary, to which it is necessary to return in the future.

Sustainable agricultural production and food industry are closely related fields. Food-processing is a process which considerably adds to the value of the primary bio-mass. The slogan of the future agriculture of Hungary will be "to produce higher value", rather than "to produce much". On this account, Hungary is interested in developing an up-to-date food industry capable of producing competitive goods. The co-operation of food-scientists and the exchange of their ideas will have a beneficial effect on the further development of the whole Hungarian agricultural sector.

The International Union of Food Science and Technology is a Scientific Associate to the International Council of Scientific Unions (ICSU). Being a member of the ICSU Executive Board, I would like to greet, at the end of my paper, the leading officials and representatives of IUFOST, wishing fruitful work to all participants of the 9th World Congress of Food Science and Technology.

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EFFECT OF ACIDIFICATION AND FERMENTATION ON THE QUALITY CHARACTERISTICS OF CANNED MUNG BEAN (*VIGNA RADIATA WILCZEC*) SPROUTS

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A study was conducted on canned mung bean sprouts acidified with one of five organic acids or by lactic acid fermentation. The quality of the processed sprouts was determined by physical, chemical, microbiological and sensory analyses after a two month storage period at room temperature.

The data obtained for colour, texture, flavour and the Overall Quality Index (OQI) indicated the possibility of processing high quality canned mung bean sprouts acidified either with citric or acetic acid. Since those data were not statistically different, acetic acid was picked out as the most favourable one due to its lower price. All the acidification procedures associated with thermal processing led to no microbiological activities on the final products after the two month incubation period, ensuring minor risk of food born diseases.

Keywords: mung bean sprouts, thermal processing, acidification, fermentation

Most of the vegetables are considered as low acid foods (FOOD & DRUG ADMINISTRATION, 1976) and should therefore be processed at high temperature to avoid development of *Clostridium botulinum* (RIVERO, 1973; ITO & CHEN, 1978). In the United States the incidence of botulism is frequently associated with the ingestion of home made preserves having pH values above 4.60 (FRAZIER, 1967; AYRES, 1975). In Brazil, GRANER and co-workers (1978) called attention to the risks represented by the canning of home preserves using recipes that do not take into consideration the final pH of such products.

Vegetables have been generally processed by high energy consuming methods of freezing or conventional canning. Considering the high costs of energy and equipment, alternative methods must be developed to reduce cost and to maintain high safety standards from the microbiological point of view, high nutritional quality and good palatability (KOSUP & SISTRUNK, 1982).

The use of acidification or lactic acid fermentation to lower the pH value of vegetables below 4.60 seems to be an alternative technique to save energy during

processing and to provide a product of acceptable quality (KOTZEKIDOU & ROUKAS, 1987; MEURER & GIERSCHNER, 1992; NOGUEIRA et al., 1994).

The purpose of this work was to evaluate the effect of acidification and fermentation and to provide information, currently lacking, on the quality of canned mung bean sprouts processed in these ways, at atmospheric pressure.

1. Materials and methods

1.1. Raw material

Samples of mung bean sprouts used in this study were commercially grown in Sao Paulo country, State of S. Paulo, Brazil.

1.2. Analyses of raw and/or processed material

1.2.1. Drained weight, pH, moisture, crude fat and ash were performed by standard methods (A.O.A.C., 1980).

1.2.2. Crude protein. Total nitrogen was determined by micro-Kjeldahl method following the technique described by BAILEY (1967) and protein was expressed as %N×5.85.

1.2.3. Total carbohydrates. Carbohydrates were extracted with 85% methanol (SOUTHGATE, 1969) and determined with anthrone reagent (NEISH, 1952).

1.2.4. Total soluble solids were determined by direct reading with a hand refractometer.

1.2.5. Sensory evaluations. Colour, texture and flavour were evaluated by ten trained panelists on a 1 to 10 points scale, with 1 being the least desirable and 10 the most desirable sample. The panelists were conducted in a room equipped with individual booths, water and indoor red and fluorescent lighting. The red lighting was utilized for evaluation of texture and flavour. Each panelist was presented with an individual sample (50 g) portioned into white cups coded with random numbers. Sensory attributes were evaluated in comparison with a non acidified control prepared at the same time as the samples evaluated, following the procedures described later for processing the acidified product.

An Overall Quality Index (OQI) was obtained by taking the average of the sum of colour, texture and flavour given to each sample.

1.2.6. Microbiological analyses were performed using two jars of each treatment or 50 g of the raw material and tested for commercial sterility according to the APAH (CORLETT & DENNY, 1984). Results were reported in terms of presence (+) or absence (–) of microbiological activity.

1.2.9. Statistical analyses. The experiment, for sensory attributes evaluation, was designed as a factorial with four treatments (three organic acids and fermentation) and two replicates. All other analyses were conducted with six replicates. The mean separation was assured by Duncan's multiple range test (PIMENTEL GOMES, 1990).

1.3. Processing

The mung bean sprouts were washed, blanched in boiling water for 1 minute and immediately cooled in tap water at room temperature. After cooling the sprouts were divided in to two lots.

The first lot was acidified by adding the right amount of either acetic, citric or lactic acid to the canning brine to reach a 4.30 equilibrium pH in the final products, as determined by acidification curves, previously obtained for each acid (ZAPATA & QUAST, 1975).

The sprouts were filled to a weight of 100 g into glass jars of 200 ml and 80 ml 1.5% NaCl solution plus 1% vitamin C (w/v) were added. The jars were sealed with epoxy lacquered aluminium lids.

The other lot was fermented at 35 °C by adding 1 g of a commercial starter (*Lactobacillus delbrueckii subsp. bulgaricus* and *Lactococcus lactis*, 1:1) to 10 l of a 1.5% NaCl solution, until products reached a 4.30 equilibrium pH and then immediately heat processed to stop fermentation.

The sprouts were filled into glass jars as described before, using the fermented brine solution.

All the filled glass jars were sealed and processed for 30 min in boiling water. After a two month incubation at 25 to 31 °C and the lighting conditions ranging from diffuse light during day-time to darkness during night-time, the final products were evaluated.

2. Results and discussion

The chemical and physical parameters of the unprocessed sprouts are as follows (an average of five replicates): $2.31 \pm 0.01\%$ crude protein; $2.28 \pm 0.4\%$ total carbohydrates; $0.09 \pm 0.01\%$ crude fat; $0.29 \pm 0.02\%$ ash; $4.20 \pm 0.06\%$ total soluble solids; $94.42 \pm 0.06\%$ moisture and 6.47 ± 0.1 pH.

The microbiological analyses indicated that the unprocessed mung bean seed sprouts possessed a very large population of viable microorganisms (Table 1). The count of mesophilic, psychrophilic and total coliform were far above of the 1.3×10^9, 2.3×10^8 and 3.2×10^6 g^{-1} values respectively, related by PATTERSON and WOODBURN

(1980) and of the 10^6 fecal coliform reported by SLY and ROSS (1982). No references concerning thermophilics could be found in the literature.

Although ANDREWS and co-workers (1982), SPLITTSTOESSER and co-workers (1983), or SPLITTSTOESSER and MUNDT (1984) had considered the high counts of non pathogenic bacteria on fresh mung bean sprouts quite normal from quality or safety aspects, the short shelf life of the product is indeed due to its high microbial contamination.

On the other hand, the larger the microbial population the higher will be the chances for pathogenic bacteria, such as *Bacillus cereus*, or *Klebsiella pneumoniae*, to occur (PTAK et al., 1973; MORTIMER & MCCANN, 1974; PORTNOY et al., 1976; SCHIEMANN, 1978; PATTERSON & WOODBURN, 1980; PARK et al., 1983; and PARK & SANDERS, 1990).

These results and the reports above indicate that the heat processing of mung bean sprouts is essential in order to offer the consumer a safe and high quality product.

Titration curve was used to determine the amount of acid necessary to acidify mung bean sprouts from pH 6.47 to pH 4.30. The results indicated that it varied according to the acid used, 0.18 citric acid, 0.21 g lactic acid and 0.24 g acetic acid per 100 g sprouts were needed to lower the pH (Fig. 1). Such differences, as very well known, were due to the buffering capacity of the sprouts and the acid dissociation constant (BERNHARDT, 1989).

Acidification with acetic or citric acid was the best treatment to maintain the colour of the product. The less acceptable treatments, concerning this parameter, were direct acidification with lactic acid and lactic acid fermentation (Table 2). These data seem to indicate a negative effect of lactic acid on colour of the processed sprouts.

Table 1

Microbial population of unprocessed mung bean sprouts

Type of microorganism	Number of microorganisms per g	
	Range of count	Average value
Mesophilic	$1.9 - 2.4\times10^{12}$	2.1
Thermophilic	$2.6 - 6.8\times10^{11}$	4.9
Psychrophilic	$4.1 - 10.0\times10^{10}$	6.7
Total coliforms	$5.4 - 16.0\times10^{8}$	10.3
Fecal coliforms	$2.4 - 5.4\times10^{8}$	3.0

(JARVIS, 1989). Based on 5 replicate determinations

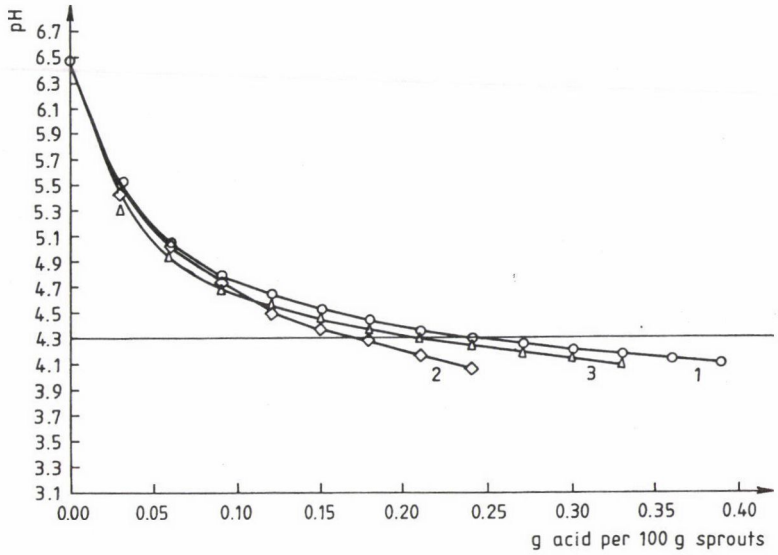

Fig. 1. Titration curves of mung bean sprouts. 1: Acetic acid; 2: citric acid; 3: lactic acid

Texture of sprouts acidified with acetic or citric acid also did not differ from the control, while acidification with lactic acid impaired it (Table 2). This fact had been reported by BUESCHER and CHANG (1983). Although not differing from the control or the samples acidified with acetic or citric acid significantly, the lactic acid fermentation affected the texture of processed sprouts negatively, too. This could be expected since lactic acid is the major end product yielded by that metabolic pathway.

Table 2

Effect of treatments on sensory characteristics and the Overall Quality Index of canned mung bean sprouts

Treatments	Colour	Texture	Flavour	OQI
Control	7.55^{ab}	8.25^{a}	5.00^{b}	6.93^{ab}
Acetic acid	7.95^{ab}	7.65^{a}	7.20^{a}	7.60^{a}
Citric acid	8.00^{a}	7.55^{a}	7.35^{a}	7.63^{a}
Lactic acid	6.65^{b}	5.80^{b}	6.45^{ab}	6.30^{b}
Fermentation	4.15^{c}	6.80^{ab}	3.05^{c}	4.67^{c}

Means with the same letter in the same column are statistically not different at the 5% level

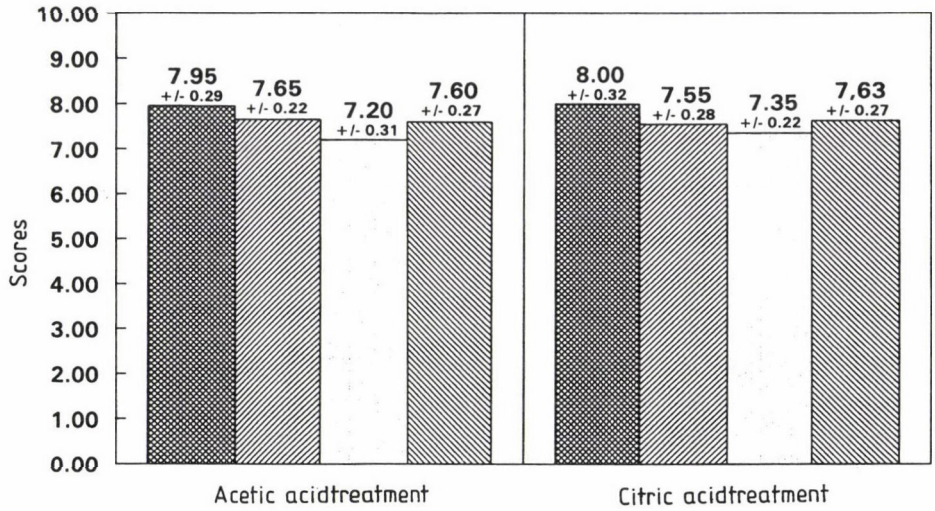

Fig. 2. Final evaluation of treatments. ▩: Colour; ▨: texture; ⊡: flavour; ▧: OQI

In general, the direct acidification improved the flavour of canned sprouts. However, fermentation adversely influenced the flavour due to development of off-flavours probably as a result of microbial cell lysis during heat processing and storage. Replacement of the fermented brine by a fresh one will certainly avoid this problem as well as the brine turbidity present in the final product.

Table 3

Chemical and physical attributes of canned mung bean sprouts

Treatments	Drained weight (g)	Total solids (%)	pH
Acetic acid	96.66^{b}	1.90^{a}	4.27^{a}
Citric acid	97.38^{b}	1.90^{a}	4.20^{a}
Lactic acid	97.77^{b}	1.63^{b}	4.26^{a}
Fermentation	106.87^{a}	1.85^{a}	4.26^{a}

Means with the same letter in the same column are statistically not different at the 5% level

The data of the Overall Quality Index (OQI) indicated acidification with lactic acid or by fermentation as the less acceptable treatments. Minor differences (statistically not significant at 5% level) were observed among acidification with acetic and citric acid and the non acidified control (Table 2). Nevertheless, these results indicated that acidification with one of the acids improved colour and flavour of processed mung bean sprouts. These sensory attributes are the most important factors for consumer's decision in selecting foods.

Since no statistical differences were observed between acetic and citric acid acidification, based on the lower price of acetic acid, this treatment was suggested as the most appropriate one for preserving mung bean sprouts (Fig. 2).

As expected, the physical and chemical attributes measured were not influenced by the treatments (Table 3). Except for fermentation, the drained weight slightly decreased in relation to that product filled into jars. Total soluble solids decreased in the processed products. Data of pH values were very close to the 4.30 previously calculated. No biological activity was detected in any of the final products after the two month incubation at 25 to 31 °C, compared to the control represented by the fresh product.

In conclusion, acidification of mung bean sprouts with citric or acetic acid resulted in a non favourable environment for the occurrence of food born diseases and yielded a final product with desirable organoleptic qualities besides the possibility of requiring less energy input and lower cost equipment, compared with the conventional retorting procedures demanded by such a low acid food.

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PROCESSING AND STORAGE EFFECTS ON THE QUALITY OF DEHYDRATED APPLES

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In this work the influence of shape (slices, sticks) and pretreatment of apples on the quality of dehydrated apples was investigated. For that purpose 3 various treatments were performed: A (blanching in water at 80 °C for 10 min), B (dipping in 40% sucrose solution + 0.1% citric acid at 60 °C for 30 min), C (dipping in 40% sucrose solution + 0.1% citric acid at 80 °C for 20 min). Drying was carried out in a cabinet air dryer. During processing chemical composition, bulk density and organoleptic properties were studied. The results obtained showed that osmodehydration caused significant increase of solid matter content primarily in apple slices. Drying process was longer for osmodehydrated apples than for the blanched sample, and it was longer for apple sticks than slices. Sensory evaluations of dehydrated apples showed that osmodehydration improved their organoleptic properties, especially taste, that remained almost the same even after 8 month of storage. Storage resulted in insignificant changes in chemical composition as well as in organoleptic properties of both apple slices and sticks.

Keywords: osmodehydration, apple, chemical composition, storage, organoleptic properties

Physico-chemcial properties and organoleptic characteristics of dehydrated products, especially fruit and vegetable, depend significantly on the drying procedure and drying conditions and also on the pretreatment process. Commonly used pretreatment is blanching that prevents enzymatic browning and acts on the replacement tissue gas with water, aqueous solutions of salts or sugar syrup.

Recently, osmodehydration procedure is more frequently used for fruit dehydration. The aim is to produce dehydrated fruits with excellent organoleptic characteristics, primarily colour and texture (KIM, 1990; TORREGGIANI, 1993; PAOLETTI & MENESATTI, 1993; QUINTERO-RAMOS et al., 1993; ADAMBOUNOU et al., 1994). Such products would uptake snack characteristics.

Most investigations were carried out with apple cut in different shapes, slices, cubes, sticks, and osmodehydrated in sugar solutions of various sucrose concentration, at different temperatures for varying times. It was pointed out that some investigated parameters have significant influence on osmodehydration. So, greater sugar

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concentration and higher temperature increase moisture loss and the uptake of sugar by the apple (KIM, 1990; VIDEV et al., 1990; SHUKLA, 1991). On the basis of the results of osmodehydration process investigation, mathematical models for predicting kinetics of osmodehydration of several products (apple, carrot) in solutions of various sugars, were developed (AZUARA et al., 1992; LENART, 1992; HOUGH et al., 1993; YAO & MAGUER, 1994). Besides osmotic dehydration at atmospheric pressure, some investigations were carried out under vacuum conditions, too. It was observed that mass transfer kinetics is much faster in vacuum operation (SHI & MAUPOEY, 1993; FITO & PASTOR, 1994). Vacuum treatments intensify capillary flow and increase water transfer ratio, but have no significant influence on sugar uptake.

The aim of our research was to investigate various kinds of treatment as well as apple's shape on both, drying process and physico-chemical and organoleptic characteristics.

1. Materials and methods

Investigations were carried out with apple variety Ida Red (*Malus domestica*) purchased from local market. Apples were washed in cold water, peeled and cut in slices 3.4×61.1×30 mm (leaf shape) and 7.5×59.3×5.5 mm (stick shape), respectively. Slices as well as sticks were blanched as follows:

A: in water at 80 °C for 10 min;
B: in 40% sucrose solution with 0.1% (m/v) citric acid, at 60 °C for 30 min (osmotic dehydration);
C: in 40% sucrose solution with 0.1% (m/v) citric acid, at 80 °C for 20 min.

Next, the apple slices and the apple sticks were dried in a laboratory cabinet air dryer constructed at the Faculty of Food Technology and Biotechnology, University of Zagreb. Dimensions of drying chamber are: length 0.2 m, height 0.37 m and width 0.2 m. Blanched apples were spread on the three wire mesh trays (dimensions 0.19×0.30 m and 0.15 m) and conventionally air dried with cross flow air at 75 °C, to obtain a final moisture content of about 5%. During process, the position of trays was changed every 15 min, to ensure the uniform drying conditions. Drying process was monitored by weighing the samples. Dehydrated apples were packed in polyethylene bags and stored for 8 month at 4.2% relative humidity (RH) (in a desiccator containing $CaCl_2$), at 20 °C. The relative humidity of air in desiccator was determined by hygrometer.

In order to investigate the influence of blanching, drying and storage on the chemical composition, some physical parameters and organoleptic properties of apples, the following analysis were carried out:

- Solid matter content was determined by drying at 105 °C to a constant mass.

- Invert and total sugar content was determined according to Luff-Schoorl's method (VAJIC, 1964). Five grams of sample were chopped, filled up to 250 cm^3 with distilled water and filtered (filtrate I). Twenty-five cm^3 of filtrate I were then mixed with 25 cm^3 Luff's reagent and cooked for 10 min. Next 10 cm^3 of 30% (m/v) KJ and 25 cm^3 of 6 M H_2SO_4 were added to the sample. The solution was titrated with 0.1 M $Na_2S_2O_3$ solution. Invert sugar content was determined from the difference of cm^3 of $Na_2S_2O_3$ solution used for sample's and blank probe titration.

For determination of total sugar content 10 cm^3 filtrate I was diluted with 30 cm^3 distilled water and 0.5 cm^3 concentrated HCl. Hydrolysis was performed by cooking the solution for 30 min. After cooling, 1 M NaOH was added to increase the pH of solution to 7. The next procedure was the same as for the invert sugar content determination.

- Total acidity was determined by potentiometric titration. Twenty-five g apple were homogenized in a blender and diluted with distilled water to 250 cm^3 in a volumetric flask. Dehydrated apples, after homogenization, were cooked for 30 min in a water bath and than diluted to 250 cm^3. Finally the solution was filtered through filter paper, and 25 cm^3 filtrate was titrated with 0.1 M NaOH to pH 8.1 using a glass electrode pH meter (MA 5740 - "Iskra", Kranj, Slovenia) at 25 °C. The results were expressed as mmols of acid per 1 g solid matter.

- The pH of samples was determined at 25 °C before potentiometric titration (using apparatus MA 5740 - "Iskra", Kranj, Slovenia).

- Bulk density was determined as a ratio between mass sample and its volume, when it was placed in a beaker of known volume, without shaking.

For the sensory evaluation scoring method with a total 20-point's scale was applied (maximum points: taste 7 points, appearance 5 points, colour and odour 4–4 points). Evaluation was carried out with 5 panelists from our laboratory. Analysis of variance was used for the evaluation of the data.

2. Results

2.1. Blanching, osmodehydration

As is was previously noted, apples cut into slices of stickes were treated and submitted to drying.

Table 1

Chemical composition and pH of apples during processing and storage (procedure A)

Phase of determination	Solid matter (%)	pH	Invert sugar (% of dry matter)	Total sugar (% of dry matter)	Acidity (mmol g^{-1} dry matter)
fresh apple	15.50	3.73	51.50	65.40	1.723
after blanching[a]					
– slices	7.10	3.51	26.80	56.20	6.535
– sticks	7.10	3.47	27.90	58.50	6.803
after drying					
– slices	94.60	3.93	32.30	66.70	0.301
– sticks	94.30	4.00	29.50	69.70	0.313
after 8 month storage					
– slices	94.90	3.82	28.28	50.50	0.342
– sticks	96.20	3.93	25.50	51.90	0.364

[a] blanching in water at 80 °C for 10 min

Table 2

Chemical composition and pH of apples during processing and storage (procedure B)

Phase of determination	Solid matter (%)	pH	Invert sugar (% of dry matter)	Total sugar (% of dry matter)	Acidity (mmol g^{-1} dry matter)
fresh apple	15.50	3.73	51.50	65.40	1.723
after osmodehydration[b]					
– slices	32.20	4.05	17.50	83.80	0.283
– sticks	26.50	3.84	24.40	90.10	0.494
after drying					
– slices	94.80	4.23	20.30	90.90	0.112
– sticks	93.60	3.92	35.50	90.10	0.150
after 8 month storage					
– slices	96.90	4.22	26.30	83.80	0.104
– sticks	97.40	3.83	36.50	81.10	0.143

[b] osmodehydration in 40% sucrose + 0.1% citric acid solution at 60 °C for 30 min

Table 3

Chemical composition and pH of apples during processing and storage (procedure C)

Phase of determination	Solid matter (%)	pH	Invert sugar (% of dry matter)	Total sugar (% of dry matter)	Acidity (mmol g^{-1} dry matter)
fresh apple	15.50	3.73	51.50	65.40	1.723
after osmodehydration[c]					
- slices	33.60	3.83	10.07	85.50	0.253
- sticks	35.10	3.66	23.14	84.40	0.265
after drying					
- slices	95.30	3.86	20.69	89.10	0.130
- sticks	92.30	3.89	30.05	81.20	0.096
after 8 month storage					
- slices	96.00	3.61	21.40	87.90	0.071
- sticks	95.30	3.73	32.61	74.70	0.089

[c] osmodehydration in 40% sucrose + 0.1% citric acid solution at 80 °C for 20 min

The results of chemical analysis have shown that blanching in water (procedure A) led to significant loss of solid matter as well as sugar content, as a consequence of their diffusion (Table 1). At the same time the acidity increased and pH value decreased. These changes were the same for both the slices and sticks. Osmodehydration (procedure B) in 40% sucrose solution at 60 °C for 30 min, caused a significant increase of solid matter content primarily in apple slices (greater contact surface between apple pieces and solution) (Table 2). At the same time, as a consequence of sugar diffusion in and out of apples, invert sugar content of apple decreased but total sugar content of apple significantly increased. This procedure lowered the acidity, too.

Osmodehydration carried out at higher temperature (80 °C) and for shorter time (20 min) (procedure C) had a greater influence on the increasing of solid matter content than procedure B (33.6 and 35.1%, respectively (Table 3). Total sugar content decreased slightly compared to procedure B.

2.2. Dehydration

Samples were dried in a cabinet air dryer at 75 °C to lower the water content in apple to ca 5%. Drying time varied from 105 to 240 min depending on the shape and pretreatment procedure of the apple (Table 4). The drying time was shorter for apple

slices, than for sticks, because of the difference in their thickness (3.4 mm wide slices; 5.5×7.5 mm sticks). Apples blanched in water dried faster than apples treated with sucrose solution (80 °C for 20 min), although their solid matter content was lower. This phenomenon is a consequence of the change in the ratio of free and bound water in apples, and therefore in osmodehydrated samples the amount of bound water was greater than in others. As its removal requires more energy, the drying time for these apples was longer.

Dehydration procedure also affected certain parameters of apples. First of all, both the invert and total sugar contents increased, which can be explained by the hydrolysis of carbohydrates (starch, pectin). Total acid content decreased because of the diffusion into the solution as well as evaporation during drying process and storage (Tables 1–3).

The bulk density of dehydrated apples varied from 116.1 to 189.4 kg m^{-3} depending on the shape of apple and blanching procedure (Table 5). Lower values of bulk density were registered for apple slices due to their size and shape. Osmodehydrated apples had greater bulk density because of the sugar diffusion into the apple during the process.

Table 4

Conditions of dehydration and total processing time

Treatment (Procedure)	Temp. (°C)	Treatment time (min)		Total processing time (min)	
		slices	sticks	slices	sticks
Blanching in water	80	10	10		
(A)				115	130
Drying	75	105	120		
Osmodehydr.	60	30	30		
(B)				210	240
Drying	75	180	210		
Osmodehydr.	80	20	20		
(C)				260	260
Drying	75	240	240		

Table 5

Bulk density of dried apples

Blanching procedure	Bulk density ($kg\ m^{-3}$) sticks	slices
A – in water (80 °C/10 min)	161.4	116.1
B – in 40% sucrose solution (60 °C/30 min)	189.3	134.1
C – in 40% sucrose solution (80 °C/20 min)	198.4	136.2

2.3. Storage

Dehydrated apples were packed in polyethylene bags and stored in a desiccator at 4.2% RH and 20 °C. Storage lasted for 8 month, followed by analysis of chemical composition and sensoric evaluation. The results (Tables 1–3) showed that the solid matter content increased slightly and the acidity remained almost unchanged. Greater changes were observed only in the invert and total sugar content. In apples blanched in water (procedure A) the relative amount of invert and total sugar decreased, that means that they have reacted with other components creating some new products (nonenzymatic browning reactions).

In osmodehydrated apples (procedures B and C) invert sugar content slightly increased (hydrolysis of sucrose), but total sugar content decreased insignificantly because of the same reasons as in procedure A (nonenzymatic browning).

2.4. Sensory evaluation

Table 6

Average scores for organoleptic properties of apple slices after dehydration and 8 month storage

Sample (Procedure)	Property Taste (7)	Appearance (5)	Colour (4)	Odour (4)	Total scores
After drying					
A	5.00	4.33	3.66	2.33	15.32
B	6.83	4.16	3.16	1.00	15.15
C	6.50	4.66	3.83	1.66	16.65
After storage					
A	5.50	4.33	3.50	1.00	14.33
B	6.16	4.00	3.00	1.00	14.16
C	6.50	4.66	3.83	1.00	15.99

Table 7

Average scores for organoleptic properties of apple sticks after dehydration and 8 month storage

Sample (Procedure)	Property				Total scores
	Taste (7)	Appearance (5)	Colour (4)	Odour (4)	
After drying					
A	3.50	4.16	3.16	2.00	12.66
B	6.51	4.00	2.00	1.00	13.57
C	6.16	4.26	3.13	2.00	15.48
After storage					
A	3.50	4.00	2.50	1.00	11.00
B	5.50	4.00	1.50	1.00	12.00
C	5.00	4.16	2.50	1.00	12.66

Sensory evaluation was done by 5 panelists from our laboratory. In Tables 6 and 7 the arithmetic mean values of scores for every sample of apple slices and sticks after dehydration, as well as after storage are presented.

It can bee seen that the best samples were those obtained by procedure C (osmodehydration at 80 °C for 20 min), and the worst were those blanched in water. Osmodehydrated apples, after panelist's evaluation, had significantly better taste than others. Their appearance was evaluated as excellent because of their unified size and regular shape. Colour of apple slices was scored very high and better than that of apple sticks. For all samples the panelists established loss of natural apple's odour.

The storage caused certain decrease of total scores for all samples, although some of them retained their excellent organoleptic properties. Apple slices produced according to procedure C were the best and the worst ones were apple sticks blanched in water.

The appearance changed the least, while other properties changed more. The taste of osmodehydrated apples remained almost the same, while the taste of other samples changed much more.

2.5. Statistical analysis

In order to demonstrate the influence of pretreatment process as well as sample's shape on the sensory evaluation of dehydrated apples, the analysis of variance and probability was executed (Tables 8, 9, 10 and 11). It was found that Fisher quotient value for apple slices was lower than the limit value ($P \leq 0.05$). Therefore, there was no significant difference between the treatments. Fisher quotient value for apple sticks

Table 8

Analysis of variance for dehydrated apple slices

Source of variance	SQ	Degrees of freedom	MQ (Variances)	F	Probability
Between samples	7.4453	2	3.72265	3.16	0.0716
Error	17.6666	15	1.17773		
Total	25.1112	17			

$F_{0.05}$ (2/15) = 3.68

was higher than the limit value, which means that the differences between the treatments are statistically significant. These, and the results of analysis of variance between shapes (Tables 10, 11) indicate the fact that the pretreatment process as well as the shape, have a great influence on the organoleptic properties of dehydrated products.

3. Conclusions

Osmodehydration caused a significant increase of solid matter content primarily in apple slices due to the diffusion of sugar into the apple tissue.

Drying time varied from 105 to 240 min depending on the apple shape and their pretreatment (blanching, osmodehydration). It was longer for osmodehydrated apples. These apples also had greater bulk density owing to the increased sugar content.

Eight month storage caused insignificant changes in chemical composition and organoleptic properties of both apple slices and sticks.

Sensory evaluations of dehydrated apples have shown that osmodehydration improved their organoleptic properties, especially taste, that remained almost unchanged even after 8 month of storage.

Table 9

Analysis of variance for dehydrated apple sticks

Source of variance	SQ	Degrees of freedom	MQ (Variances)	F	Probability
Between samples	14.8	2	7.4	7.65	0.00723
Error	11.6	12	0.9666		
Total	26.4	14			

$F_{0.05}$ (2/12) = 3.88

Table 10

Average total scores of dehydrated apple slices and sticks

Apple's shape	Sample A	Sample B	Sample C
Slices	15.5	15.2	16.7
Sticks	12.4	12.6	14.6

Table 11

Analysis of variance of results from Table 10

Source of variance	SQ	Degrees of freedom	MQ (Variances)	F	Probability
Between samples	3.97	2	1.935	15.88	0.060
Between shapes	10.14	1	10.140	81.12	0.012
Error	0.25	2	0.125		
Total	14.36	5			

$F_{0.05}$ (2/12) = 19.00

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EFFECTS OF MICROWAVE HEATING ON THE CHEMICO-NUTRITIONAL VALUE OF SOYBEANS

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The effect of microwave heating (ν=2450 MHz) on the quality of the raw non-moistened and moistened soybeans was examined by measuring the changes in the antinutritive constituents (trypsin inhibitor and urease), protein (nitrogen solubility index NSI), amino acid profile and brown pigments (development of melanoidins).

Comparing the quality parameters observed, it has been concluded that moistening before microwaving ensures the soybeans higher quality.

Keywords: soybeans, microwave heating, moistening, chemico-nutritional value, trypsin inhibitor, urease activity

Soybeans are potentially valuable protein sources with appreciable content of high quality fat and a beneficial mineral-vitamin pattern.

The possibility of using raw fullfat soybeans as an exceptional ingredient rich in protein for animal diet is limited by the presence of numerous antinutritive factors (the inhibitors of proteolytic enzymes, trypsin and chymotrypsin, phytates, saponins, etc.). The high content of these constituents, first of all that of the trypsin inhibitor, decrease the growth of rats, poultry and other experimental animals (LIENER & KAKADE, 1980). RACKIS and co-workers (1986) reported that the high trypsin inhibitor content of raw soybeans dominantly inhibit the proteolytic action of the pancreatic enzyme trypsin.

The inactivation of thermolabile trypsin inhibitor and other heat sensitive antinutritive constituents of raw soybeans is achieved through different types of heat treatments like roasting, micronization, extrusion, hydrothermal treatment, etc. (THOMPSON, 1987).

The microwave heating is a new possible way for raw soybeans treatment. The effect of treatment at 2450 MHz frequency (microwave region) (HAFEZ et al., 1983; SAKLA et al., 1988; POUR-EL et al., 1981) and that of the dielectric heat treatment (27.12 MHz) (PETRES et al., 1990) on the physico-chemical and nutritional properties of soybeans were studied. In these investigations microwaves had a beneficial effect on soybean quality resulting in a decrease in the antinutritive constituent.

The contents of trypsin inhibitor and hemaglutinin are used as indicators of the quality of soybeans treated, that is, of heating adequacy and other parameters were examined to control the heat destruction of proteins (nitrogen solubility index (NSI), protein dispersibility index (PDI), amino acid profile, lysine availability). The assay for urease activity may be useful in determining whether soybeans have been adequately treated (MCNAUGHTON, 1981), since CASKEY and KNAPP (1944) have pointed out that heat destruction of urease is positively correlated with the decrease in trypsin inhibitor content.

The high content of the reactive amino acids in soybeans (lysine and arginine) accompanied by a considerable content of sugar, under the conditions of the increased temperatures, lead to Maillard's reactions, the final products of which are brown pigments – melanoidins. The increase of melanoidin content with the concomitant colour change may also be an indicator of the treatment adequacy, since overaggressive treatment causes undesirable intensive darkening (high melanoidin concentration) accompanied by a decrease in the nutritional value of the final product.

The objectives of this research were to evaluate the influence of microwave heating on the soybeans grown in the local area as a contribution to the development of new method for treating soybeans.

1. Materials and methods

The soybeans (*Glycine max* L) with the following approximate composition were used to investigate the effect of the microwave heating (ν=2450 MHz): 7.53% moisture, 40.06% crude protein, 5.98% crude fibre, 21.43% crude fat, 4.77% ash, 20.23% N-free extract, trypsin inhibitor (TI) 51.26 mg per g in dry matter, urease activity 5.16 mg N $g^{-1}min^{-1}$ at 30 °C. The soybeans were grown in the area of the North Backa (Vojvodina) in 1994.

Soybean samples (250 g) were placed on trays at a thickness of 2.5 cm. They were treated at ν=2450 MHz for 3, 5 and 7 min, respectively. The effective power of microwaves was 390 W.

The temperature of the mass was measured by a laboratory thermometer after the treatment.

In order to investigate the effect of premoistening on the characteristics of microwaved soybeans, water was added to the soybeans to achieve a moisture content of 19.52% in all treatments.

The chemical composition of soybeans (moisture, crude protein, crude fibre, crude fat, minerals and N-free extract) was determined according to A.O.A.C. (1984) methods.

Urease activity was determined according to International Standards 5506 (INTERNATIONAL STANDARDS ORGANIZATION, 1978) and trypsin inhibitor content by the method described in the work of HAMERSTAND and co-workers (1981).

Nitrogen solubility index (NSI) was determined according to A.O.C.S. (1973) method.

Melanoidin type brown pigments were determined using a model system as standard substance. The standard substance was obtained by the reaction of 0.5 mol l^{-1} glucose and 0.25 mol l^{-1} aspartic acid at pH=2.3 and at temperature of 93 °C for 230 h (MILIC et al., 1975).

Amino acid composition of the raw and heat treated soybeans (moistened and non-moistened, t=7 min) was determined with a Biotronic, Model LC 5001 analyzer. The samples were hydrolyzed prior to analysis with 6 mol l^{-1} HCl for 23 h at 110 °C. Cystine and methionine were oxidized with performic acid (at 2 °C for 15 h) (MOORE, 1963).

2. Results

In order to decrease the content of antinutritive constituents, raw soybeans were treated with microwaves (ν=2450 MHz). Urease activity and trypsin inhibitor content – indicators of the heating adequacy – of the starting and treated soybeans are shown in Table 1.

NSI value as the direct indicator of the changes in proteins and the content of brown pigments – melanoidins as an indirect indicator are shown in Table 2.

The amino acid profile of the raw and treated soybeans given in Table 3 offers a more complete understanding of protein changes.

3. Conclusions

Based on the results given in the Table 1 it can be concluded that the urease activity of the raw soybeans (5.16 mg N $g^{-1}min^{-1}$ at 30 °C) was slightly lower than usually (PETRES et al., 1990), and the trypsin inhibitor content (51.26 mg per g in dry matter) exceeded the 20–30 mg per g range (MONARI, 1988; HERKELMAN et al., 1991; BOZOVIC et al., 1992), but they were still in accordance with the findings of CAMACHO and co-workers (1981) (59.7 mg per g) and PEER and LEESON (1985) (58.6 mg per g).

Microwaving reduces the urease activity and trypsin inhibitor content, the rate of reduction being a function of treatment time (Table 1). Microwave treatment of raw soybeans for 3 min was inadequate, while the 5 and 7 minute treatment times seemed

to be adequate to reduce the urease activity. The 7 min treatment time was ideal when the trypsin inhibitor was also considered. These conclusions result from the findings that the 0.1–0.5 mg N $g^{-1}min^{-1}$ range is acceptable for urease activity, i.e., the 0.1–0.3 mg N g^{-1} min^{-1} range is the best for soybeans with the concomitant trypsin inhibitor level of 4–5 mg g^{-1} provided by adequately adjusted heat treatment conditions (MONARI, 1988).

Having in mind that the NSI value of 12.5% is considered as a result of excessive heating and that of 25.1% as a result of adequate processing, the 5 and 7 min treatment times seem to be acceptable (Table 2).

Moistening the soybeans (19.52% moisture content) before microwave treatment was performed for better heat transfer and the protection against heat destruction. The differences in urease activity, trypsin inhibitor content and NSI value between the non--moistened and moistened soybeans microwaved for the same time are statistically not significant, except for the urease activity of soybeans treated for 3 min ($P \leq 0.05$), although MCNAUGHTON (1981) reported better inhibition of the antinutritive constituents by autoclaving at 120 °C with the increase of the moisture content.

Amino acids, especially lysine (Table 3) were destructed during microwave treatment. Similar lysine destruction by dielectric treatment was recorded by PETRES and co-workers (1990).

The differences in melanoidin content are high that can be even visually recorded comparing the colour of treated soybeans. The colour varies from yellow (raw soybeans), golden yellow (shorter exposition to microwaves) to yellow-brown (7 min without moistening) that is markedly darker than the colour of soybeans moistened and treated for 7 min. The lower melanoidin content in moistened soybean is quite logical since the temperature level of these treatments is lower and it is also known that higher moisture content does not favour Maillard's reaction (MILIC et al., 1988). The melanoidin content of microwaved soybeans, regardless to the differences noticed, can be tolerated since they render visually acceptable products.

The differences in melanoidin content and in amino acids first of all in lysine of non-moistened and moistened soybeans treated for the same period are statistically significant ($P \leq 0.05$) that justifies the moistening of soybeans before microwave treatment.

Table 1

Urease activity and trypsin inhibitor content of the raw and microwaved soybeans

Sample	Moisture content		Duration of treatment at 2450 MHz (min)	Final temperature[a] (°C)	Urease activity[a] ($mgN\ g^{-1}min^{-1}$)	Trypsin inhibitor[a] (mg per g D.M.)
	before treatment (%)	after treatment[a] (%)				
R. S.	7.53	–	–	–	5.16	51.26
T. S.	7.53	7.06 ± 0.04	3	90.5 ± 0.4	2.64 ± 0.07	31.31 ± 0.79
T. S.	7.53	4.75 ± 0.06	5	127.0 ± 0.8	0.45 ± 0.04	9.28 ± 0.37
T. S.	7.53	3.59 ± 0.07	7	143.5 ± 1.9	0.27 ± 0.07	4.58 ± 0.45
T. P-M. S.	19.52	17.13 ± 0.10	3	90.0 ± 0.8	2.06 ± 0.07	29.76 ± 0.64
T. P-M. S.	19.52	13.31 ± 0.40	5	102.5 ± 1.1	0.40 ± 0.02	9.50 ± 0.34
T. P-M. S.	19.52	9.05 ± 0.14	7	113.0 ± 1.2	0.22 ± 0.03	4.27 ± 0.14

R. S.: raw soybeans; T. S.: treated soybeans;
T. P-M. S.: treated pre-moistened soybeans;
[a]: means and standard deviation of triplicate replications

Table 2

Nitrogen solubility index (NSI) and content of melanoidin of the raw and microwaved soybeans

Sample	Duration of treatment at 2450 MHz (min)	Nitrogen solubility index (NSI)[a] (%)	Melanoidin content[a] (%)
R. S.	–	65.62	–
T. S.	3	42.64 ± 2.67	0.12 ± 0.01
T. S.	5	21.74 ± 1.06	0.14 ± 0.02
T. S.	7	14.01 ± 0.18	0.35 ± 0.03
T. P–M. S.	3	44.88 ± 2.82	0.05 ± 0.01
T. P–M. S.	5	22.54 ± 0.58	0.07 ± 0.01
T. P–M. S.	7	14.67 ± 0.36	0.17 ± 0.03

R. S.: raw soybeans; T. S.: treated soybeans;
T. P–M. S.: treated pre-moistened soybeans;
[a]: means and standard deviation of triplicate replications

Table 3

Amino acid composition of the raw and microwaved soybeans

Amino acids (g per 100 g crude protein)	Raw soybeans	Treated soybeans (t=7 min)[a]	Treated pre-moistened soybeans (t=7 min)[a]
Lysine	6.10	4.83 ± 0.11	5.68 ± 0.15
Methionine	1.27	1.08 ± 0.04	0.75 ± 0.04
Cystine	2.67	2.00 ± 0.09	2.52 ± 0.09
Threonine	4.59	4.39 ± 0.08	4.40 ± 0.08
Serine	5.04	4.93 ± 0.10	5.20 ± 0.05
Aspartic acid	12.00	12.10 ± 0.18	12.82 ± 0.26
Glutaminic acid	14.73	12.71 ± 0.21	13.12 ± 0.20
Proline	4.87	4.53 ± 0.10	4.80 ± 0.10
Glycine	4.92	3.56 ± 0.08	4.32 ± 0.11
Alanine	3.99	4.03 ± 0.06	3.80 ± 0.06
Valine	5.14	3.73 ± 0.02	4.32 ± 0.08
Isoleucine	4.27	3.68 ± 0.05	3.95 ± 0.01
Leucine	7.00	6.04 ± 0.11	6.50 ± 0.08
Tyrosine	4.49	4.23 ± 0.08	3.78 ± 0.07
Phenylalanine	5.18	4.36 ± 0.03	4.80 ± 0.07
Histidine	2.75	2.84 ± 0.04	2.77 ± 0.05
Arginine	7.90	6.51 ± 0.11	7.63 ± 0.12

[a]: means and standard deviation of triplicate replications

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RHEOLOGICAL CHARACTERISTICS OF CREAMS

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The aim of this work was to investigate physico-chemical changes of semisolid food systems such as creams. For this purpose visual observations as well as rheological characteristic measurements were performed during a 28 days period at –14 °C (259 K).

Gelation, capillarity and water retention characteristics having influence on rheological properties of semisolid systems were determined.

The results show that processing, composition and type of powder substances as well as the dispersion medium (water or milk) have important role in the formation of the rheological parameters in the beginning and during storage.

The time of cream formation is shorter in water than in milk but the samples prepared with water loose their concistency faster during storage. The reason for such behaviour is the faster separation of water from their structure.

Flow behaviour index (n) and consistency coefficient (a) obtained mathematically and graphically are in good correlation.

Keywords: cream, semisolid food system, rheological properties, cream stability

Semisolid food systems are present in different food industries. The major use of these products is in the semi-prepared foodstuff production. Research in this field includes the methods and conditions of their production, the problems with composition and storage conditions as well.

Cream stability can be determined by the investigation of rheological, electrical, organoleptic and other properties.

It was found earlier that there is a good correlation between cream stability and their rheological characteristics (MANN, 1993; SINGHAL & KULHARNI, 1990, 1991; and ĐAKOVIĆ, 1972).

Semisolid systems have to retain their original organoleptic characteristics, i.e. quality and stability, for a certain period of time (MANN, 1993; SINGHAL & KULHARNI, 1990, 1991).

Cream powder consists of several different constituents. The main component is starch present at high ratio thus affecting physico-chemical and organoleptic

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characteristics of these products (SINGHAL & KULHARNI, 1990, 1991; ĐAKOVIĆ, 1972; HLYNKA, 1972; KOKINI et al., 1992 and KARLSON, 1988).

Apart from starch characteristics, physico-chemical, rheological and visual properties of creams will also be affected by the product homogeneity, the way of processing, storage conditions, pH value, cream constituents as well as dispersion medium. The above mentioned parameters have been analyzed by many authors (SINGHAL & KULHARNI, 1990, 1991; ĐAKOVIĆ, 1972; KOKINI et al., 1992 and KARLSON, 1988).

It was found that different cream parameters have approximately identical starch particle diameters but different viscosity value, which can vary. This difference strongly depends on media used for the reconstitution, i.e., milk or water (SINGHAL & KULHARNI, 1990, 1991). The homogeneity of milk itself has a great influence on viscosity value as well (KOVAČEVIĆ & TUĐA, 1982, HANSEN et al., 1991, BILIADERIS & ZAWISTOWSKI, 1990, PETRIČIĆ, 1984 and LALIĆ & BERKOVIĆ, 1989).

During milk homogenization process the diameter of fat droplets diminish by 1/10 of the original size causing an increase in viscosity. The size of fat droplets has a great influence on the rheological and visual properties of prepared creams. By definition milk is milkfat emulsion in the water with fat being suspended into fat droplets. Apart from the fat, milk consists of a number of dispersed colloidal particles which also participate in the rheological and visual properties of creams (LALIĆ & BERKOVIĆ, 1989).

Due to the formed structural changes, as a result of association force action, it is very often difficult to determine physico-chemical parameters of the cream powder products. All these changes will also affect plasticity and elasticity properties of the creams (SINGHAL & KULHARNI, 1990, 1991).

1. Materials and methods

Three different creams prepared according to manufacturer's instruction with water or milk, were studied:

- "Galetta" - cream powder with vanilla flavour, aimed for custards and creams preparation produced by "Podravka", Koprivnica
- "Šlag-krema" - chocolate flavoured foam cream produced by "Podravka" - Maribor
- "Kremin" - chocolate flavoured cream produced by "Kolinska" - Ljubljana.

Table 1 shows the main components of instant creams.

Table 1

Composition of instant creams

Kremin	Galetta	Šlag-krema
Dry matter, 12%	Dry matter, 7%	Dry matter, 7%
Fats, 25%	Fats, 25%	Fats, 25%
Total sugar, 60%	Cacao, 4.3%	Cacao, 5%
Additives,	Total sugar, 60%	Total sugar, 60%
Emulsifier,	Mono and	Gelatine,
Stabilizer, 3%	diglycerides, 3.7%	Emulsifier, 3%

Preparation procedure is generally based on reconstituting a dry mix which contains 2.8% starch or modified starch, with cold milk or water without cooking.

The samples were prepared and stored in volumetric glass cylinders enabling visual observations of changes. Samples used for rheological characteristic determination were stored in plastic cups for 28 days at -14 °C (259 K). Preparation time (15 min) as well as rotation speed (6000 r.p.m.) were identical for all of the investigated samples.

Viscosity determination was performed immediately after sample preparation as well as after 7, 14, 21 and 28 days of storage at -14 °C (259 K). Prior to measurement all samples were defrosted and tempered to the measuring temperature (20±0.5 °C). Afterwards, phase separation was observed visually.

Viscosity determination was performed on a rotary viscometer (Brookfield Syncro Lectric, U.S.A.) by measuring the angle of rotation in dependence on speed of rotation.

The values provided by the instrument were converted to viscosity using tables supplied by the manufacturers (SINGHAL & KULHARNI, 1990, 1991; HLYNKA, 1972 and LALIĆ & BERKOVIĆ, 1989).

Viscosity was determined using the following equation:

$$\eta = \alpha F \tag{1}$$

where:

η : viscosity (Pa s)

α : angle of rotation

F : factor extracted from the tables

Shear stress was calculated according to the equation (2):

$$\tau = \eta D \tag{2}$$

where:

D : shear rate (s^{-1})
τ : shear stress (Pa)
η : viscosity (Pa s).

Factors related to pseudoplastic flow were determined graphically and also calculated by applying the equation:

$$\tau = K D^a \quad (3)$$

where:

K : consistency coefficient
a : constant determining Newtonian and non-Newtonian flow behaviour
D : shear rate.

Then constant "n" is calculated:

$$\eta = K/D^n \quad (4)$$

where:

n : flow behaviour index
K : fluid consistency coefficient
D : shear rate (s^{-1})
η : viscosity (Pa s).

2. Results and discussion

The results of determination, for all of the investigated samples, are expressed as consistency coefficient ("K"), and as flow behaviour indexes "n" and "a", values obtained by both mathematical (R) and graphical (g) methods.

The results are shown in Tables 2–4.

In this paper the influence of medium (water or milk) together with the composition of powders used for preparation cream samples was examined. The dispersion medium directly influences the swelling of starch particles.

In creams such as Kremin, which are prepared with water instead of milk, starch particles swell faster because water diffuses more rapidly to the starch structure than milk. Compared to Kremin, Galetta and Slag-krema creams prepared with milk have

Table 2

Rheological parameters of samples "Galetta" as a function of storage time

	Storage time (days)	$^{n}_{R}$	$^{a}_{R}$	K_R (mPas^n)	n_g	a_g	K_g (mPas^n)
Upward curve	fresh	0.524	0.457	20.763	0.527	0.473	20.370
	7	0.648	0.315	14.594	0.662	0.338	14.256
	14	0.647	0.352	19.235	0.651	0.349	19.986
	21	0.628	0.371	31.460	0.635	0.365	31.695
	28	0.684	0.315	14.954	0.662	0.338	14.689
Downward curve	fresh	0.470	0.530	14.468	0.475	0.525	14.689
	7	0.507	0.492	7.224	0.533	0.467	7.603
	14	0.552	0.447	13.706	0.545	0.455	13.740
	21	0.514	0.485	23.708	0.510	0.490	23.823
	28	0.507	0.492	7.224	0.533	0.467	7.603

R: mathematical, g: graphical approach,
a: flow behaviour index

low viscosity values. This property is due to the amplitude of milk molecules and their high content of colloidal particles and fat micellas.

All the examined samples prepared with water and milk show thixotropic loop which is in Kremin creams accompanied by antithixotropic loop.

Table 3

Rheological parameters of samples "Slag-krema" as a function of storage time

	Storage time (days)	$^{n}_{R}$	$^{a}_{R}$	K_R (mPas^n)	n_g	a_g	K_g (mPas^n)
Upward curve	fresh	0.721	0.278	40.788	0.718	0.282	41.591
	7	0.574	0.425	36.53	0.544	0.456	35.399
	14	0.598	0.401	277.671	0.604	0.396	277.332
	21	0.477	0.527	159.068	0.568	0.432	172.583
	28	0.624	0.375	210.358	0.641	0.359	207.969
Downward curve	fresh	0.679	0.320	34.481	0.652	0.348	33.651
	7	0.575	0.424	36.458	0.538	0.462	34.833
	14	0.472	0.527	225.42	0.501	0.499	231.206
	21	0.481	0.581	153.814	0.462	0.538	154.211
	28	0.580	0.420	162.314	0.421	0.579	148.936

Table 4

Rheological parameters of samples "Kremin" as a function of storage time

	Storage time (days)	$^{n}_{R}$	$^{a}_{R}$	K_R (mPas^n)	n_g	a_g	K_g (mPas^n)
Upward curve	fresh	0.775	0.224	215.358	0.729	0.271	233.883
	7	0.514	0.485	29.123	0.514	0.485	29.174
	14	0.532	0.467	39.558	0.563	0.437	46.989
	21	0.769	0.230	33.719	0.803	0.196	32.982
	28	0.500	0.500	10.507	0.523	0.477	11.587
Downward curve	fresh	0.585	0.414	181.733	0.438	0.562	170.608
	7	0.530	0.470	24.733	0.504	0.496	22.594
	14	0.610	0.389	40.181	0.583	0.417	34.040
	21	0.532	0.467	20.244	0.571	0.428	21.129
	28	0.296	0.703	5.145	0.317	0.683	5.861

According to the shape of flow curve (τ, D and logτ, logD) (Figs. 1, 2 and 3) creams show Keson's type plastic flow as a consequence of interaction between particles and dispersion medium.

Results obtained for three types of cream samples prepared with water (Kremin) and milk (Galetta, Slag krema) are given graphically (Figs. 1, 2 and 3) and in Tables (2, 3 and 4). They show that procedure of sample preparation strongly affects the initial rheological value, and the visual characteristics of semisolid type of creams are strongly affected by the type of cream powder.

For all of the investigated samples thixotropic behaviour was observed. The effect is the result of cream powder composition and dispersion medium. It can be explained on the basis of starch molecule characteristics, in dependence on temperature (15–20 °C) as well as on the physico-chemical properties of dispersion medium.

Freshly prepared samples, characterized with free linear molecules, show linear Brownian motion within solution entrapped into a gel. Upon ageing mobile groups, capable to form side bonds, interact with neighbouring chain macromolecules causing macromolecule network to shrink thus increasing the level of a gel crystallisation while a space containing entrapped liquid phase decreases. Furthermore, ostentation process decreases and water liberation from the product occurs.

Cream formation is faster when using water as dispersion medium instead of milk.

As it was already mentioned, the homogeneity of the constituents is the most important factor which affects cream viscosity. Therefore, the better homogeneity performed the highest viscosity thus a better stability of the products achieved (SINGHAL & KULHARNI, 1990, 1991 and HLYNKA, 1972).

During storage syneresis of the cream, i.e., water release from the capillary parts of the starch, occurs. It has a great influence on the rheology and visual appearance of the cream. Therefore, creams can be considered as lipophilic gels able to release water from a gel by no external force, i.e., by internal structure rearrangement.

As starch particles are not homogeneous the separation of starch constituents having different physico-chemical characteristics occurs (LALIC & BERKOVIC, 1989).

During storage, due to water liberation from its texture, creams reconstituted with water are characterized with lower shelf-life compared to those reconstituted with milk.

A good correlation between "a" and "n" values obtained graphically and mathematically (Tables 2–4) is achieved.

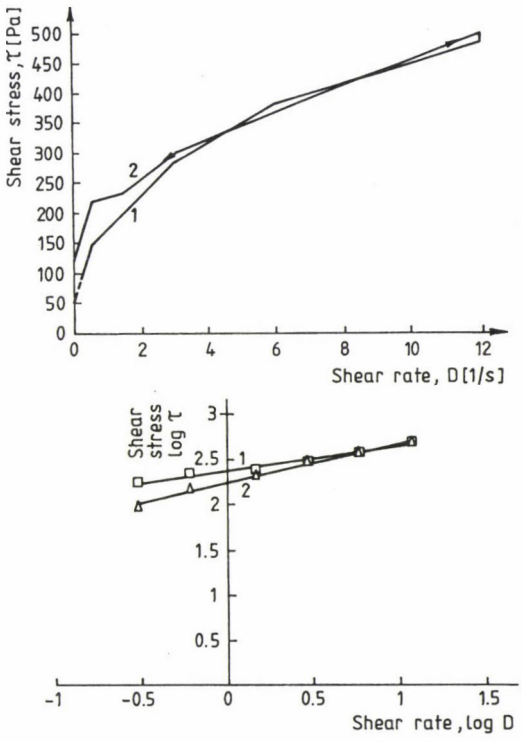

Fig. 1. Shear stress values in dependence on shear rate for the fresh "Kremin" sample. 1: Data on upward curve; 2: data on downward curve

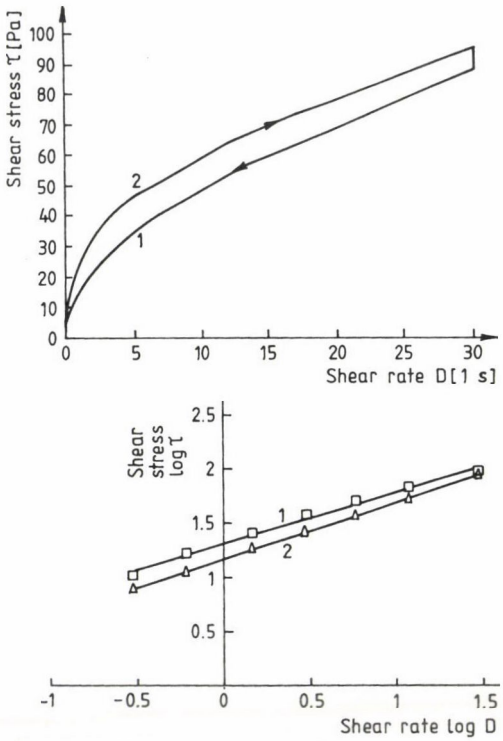

Fig. 2. Shear stress values in dependence on shear rate for the fresh "Galetta" sample. 1: Data on upward curve; 2: data on downward curve

3. Conclusions

Based on measurements the following conclusions can be drawn:

- The investigated creams were fluids showing plastic flow behaviour.

- "Šlag-krema" samples had the best rheological and visual characteristics, while "Kremin" showed the lowest stability and quality as well.

- Although samples were stored at low temperature, changes with time occurred. Values of "n" and "a" for the "Kremin" samples reconstituted with water showed the greatest changes. Constant "K" gradually decreases which imply that viscosity decreases with time. "Galetta" samples haven't changed significantly during storage.

- For the "Galetta" and "Slag-krema" samples the coefficients "a" and "n" did not show an oscillation in values observed for the "Kremin" samples during storage at -14 °C (259 K).

- Samples reconstituted with water showed the lowest stability. Shelf-life of 28 days was obtained for the "Slag-krema" samples, while a shelf-life of 15 and 7 days was achieved with "Galetta" and "Kremin" samples, respectively.

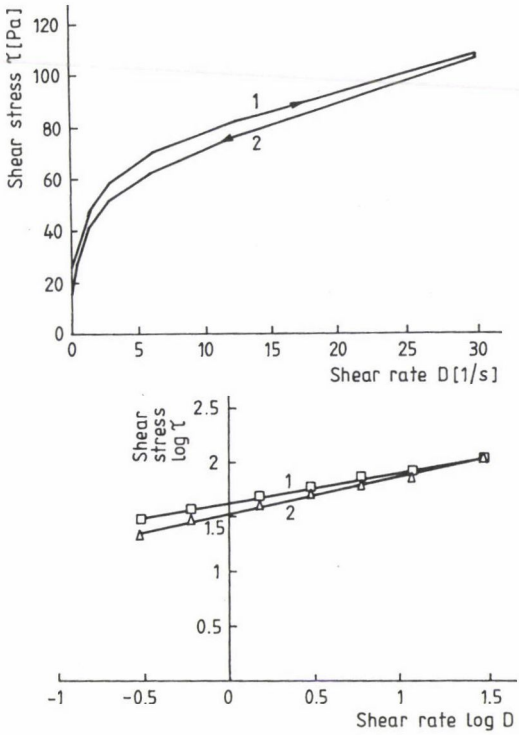

Fig. 3. Shear stress values in dependence on shear rate for the fresh sample "Šlag krema". 1: Data on upward curve; 2: data on downward curve

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SHORT COMMUNICATION

EXTENSION OF SHELF-LIFE OF A VACUUM-PACKAGED CHILLED MEAT PRODUCT BY COMBINATION OF GAMMA RADIATION, ASCORBIC ACID AND SODIUM LACTATE*

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Introduction

Various studies demonstrated that low doses of ionizing radiation which do not change the sensory quality and physical state of the product can extend considerably the shelf-life of specific refrigerated foods (URBAIN, 1986; WHO, 1988). Therefore, exploring the possibilities of using ionizing radiation in combination with environmental stress factors may significantly contribute to the development of new types of combination processes and new types of preserved foods.

Procedure

Shelf-life of a vacuum-packaged, ready-to-fry, minced meat product, 'tenderloin rolls' (FARKAS & ANDRÁSSY, 1993), was studied as affected by 2 kGy gamma radiation dose, reduction of pH (from 6.1 to 5.6) by ascorbic acid (AsH), reduction of water activity (from 0.975 to 0.962) by sodium lactate, and their combinations. Experimental batches were stored at +2 °C for 4 weeks, then samples were transferred into a +10 °C refrigerator for an additional week to simulate household conditions after purchase. Total plate counts (TPC), counts of presumptive lactobacilli (LB), the

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Enterobacteriaceae (EB), and sulphite-reducing clostridia were estimated periodically. Changes of pH during storage were also followed. Comparative estimation of sensory qualities, thiamin contents, and thiobarbituric-acid reactive substances (TBA-values) were also carried out. Sensory testing of the fried samples was performed by an 11--member panel using a 9-score hedonic-scale and Kramer's rank tests on taste, smell, texture and colour.

Results

Microbiological shelf-life was estimated as a number of days elapsed until the total aerobic viable cell counts reached the level of 10^8/g. Both pH-reduction (by ascorbic acid) and a_w-reduction (by Na-lactate), or their combined application, slowed down the microbial growth, however, in spite of the extension of shelf-life the microbiological safety of these experimental batches could not be considered as fully assured by the additives only, because at the end of the above defined shelf-life the Enterobacteriaceae counts amounted to the order of magnitude of 10^5 g^{-1} level in samples containing both ascorbic acid and Na-lactate.

The irradiation with 2 kGy radiation dose could reduce the TPC by approx. 99%, and the Enterobacteriaceae count by about four log cycles, and extended the microbiological shelf-life of the vacuum-packaged chilled meat product by a factor of at least three. The combined effect of pH reduction plus irradiation, or, a_w-reduction plus irradiation expressed itself in the extension of the lag phase of bacterial growth, while microbiological stability of the product was maintained during the five weeks of storage after the triple combination (Fig. 1). Combination treatments prevented growth of Enterobacteriaceae even at 10 °C incubation at the end of storage. Sulphite-reducing clostridia were always under the detection level (< 10 g^{-1}).

Thiobarbituric acid (TBA) values were not significantly changed neither by irradiation nor by the 5-week refrigerated storage. Means of TBA-values remained in the low range of 0.35 to 0.86 mg/kg at each testing. The thiamin level (approx. 1.2 mg/kg in the control samples) was reduced by approx. 33% as an effect of the 2 kGy gamma radiation. However, samples containing ascorbic acid and/or Na-lactate lost only approx. 10% of their thiamin content as an effect of the irradiation. Ascorbic acid containing samples tended to receive somewhat higher, but statistically non-significantly different colour score than other samples. The radiation dose of 2 kGy alone, or, in combination with additives did not significantly affect the sensorial acceptability of the product, although average scores on odour and taste of irradiated and acidified samples tended to obtain somewhat lower scores.

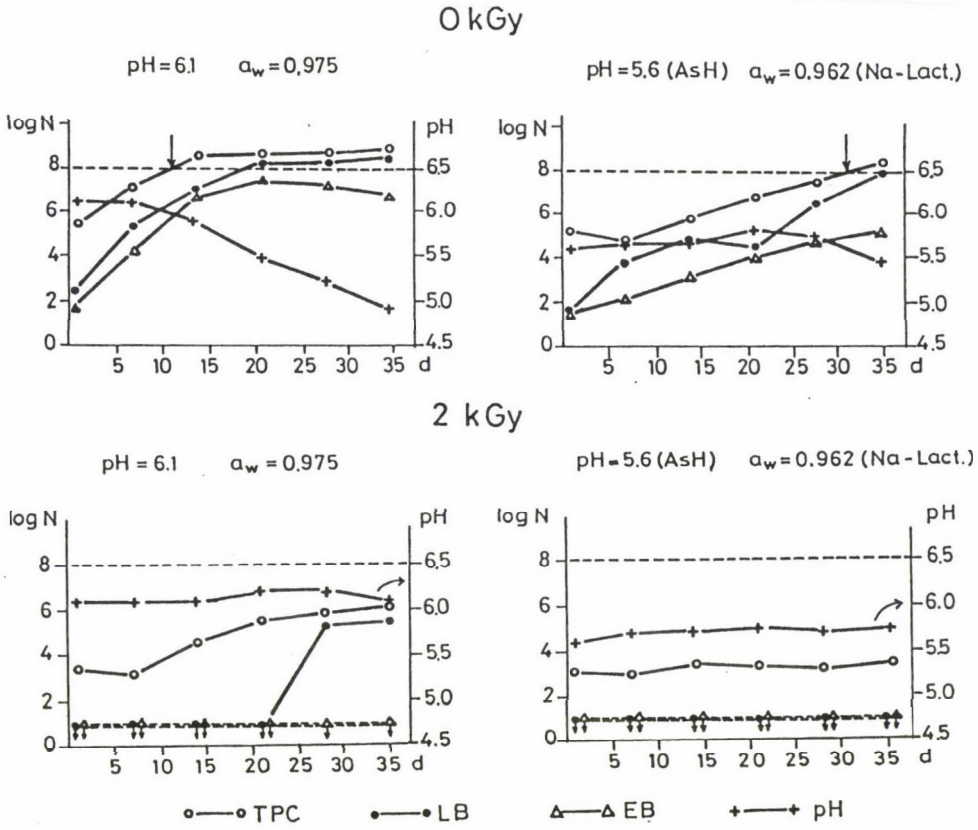

Fig. 1. Microbiological- and pH-changes of unirradiated (0 kGy) and irradiated (2 kGy) samples during storage for four weeks at +2 °C, followed by one week at +10 °C. TPC: total aerobic plate count; LB: lactic acid bacteria; EB: Enterobacteriaceae; ASH: ascorbic acid; Na-Lact.: sodium-lactate

Conclusions

It is possible to increase considerably the shelf-life of vacuum-packaged chilled product studied with a sensorially acceptable radurizing dose in combination with slight reduction of pH and water activity. Our results confirm observations of previous

studies that the presence of lactic acid bacteria represents a sort of 'built-in' safety factor in this and similar products (HOLZAPFEL, 1989; MATILLA-SANDHOLM & SKYTTÄ, 1991). Although they grow slowly enough permitting thereby a reasonably extended shelf-life of the combined-treated chilled product, they produce a decreasing pH – eventually, specific antimicrobial substances, too – during longer-term storage, or, more quickly, at temperature abuse of the preserved product, which could affect adversely the growth of harmful components of the microflora.

*

These studies have been supported by a grant of the National Scientific Research Foundation (OTKA 1/3-611) and the Research Contract No. 6271/RB of the International Atomic Energy Agency.

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BEHAVIOUR OF *LISTERIA MONOCYTOGENES* IN AN EXTENDED SHELF-LIFE CHILLED MEAT PRODUCT*

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Objective and procedure

Shelf-life of a refrigerated, vacuum-packaged, ready-to-fry, minced meat product, 'tenderloin rolls', can be considerably extended by combining slight reductions of its pH (by ascorbic acid, AsH) and water activity (by Na-lactate) with a gamma radiation dose of 2 kGy (FARKAS, 1994). It is important, however, that extended shelf-life, minimally processed chilled foods shall maintain also their microbiological safety, with particular reference to those food-borne pathogens which are capable of growth at refrigeration temperatures. *Listeria monocytogenes* is one of such microorganisms. It grows at refrigeration temperatures, is enhanced by decreased oxygen and elevated carbon dioxide levels, and is tolerant of salt (SKOVGAARD, 1989). Therefore, in order to assess the microbiological safety of the aforementioned extended shelf-life chilled product, *Listeria monocytogenes* 4ab No.10 was inoculated into the product at 10^3 and 10^4 per gram inoculum levels, resp., before radiation treatment and microbiological changes of experimental batches were followed during post-irradiation chilled storage. Packaging, treatments, storage conditions and investigations of the spoilage microorganism were the same as described in our previous papers (FARKAS, 1994; FARKAS et al., 1995). The viable cell counts of *Listeria* was estimated by the MPN technique using sub-culturing five replicates at various dilution levels in OXOID *Listeria* enrichment broth and the presence of the test organism was detected on *Listeria*-selective agar plates ('Oxford formulation'). The dilution levels were

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considered as *Listeria*-positive, when – due to the aesculin-hydrolysis – the 6,7-dihydroxycoumarin was reacting with the ferric salt, creating a dark brown colour. The catalase-positivity of suspected colonies was also checked.

Results

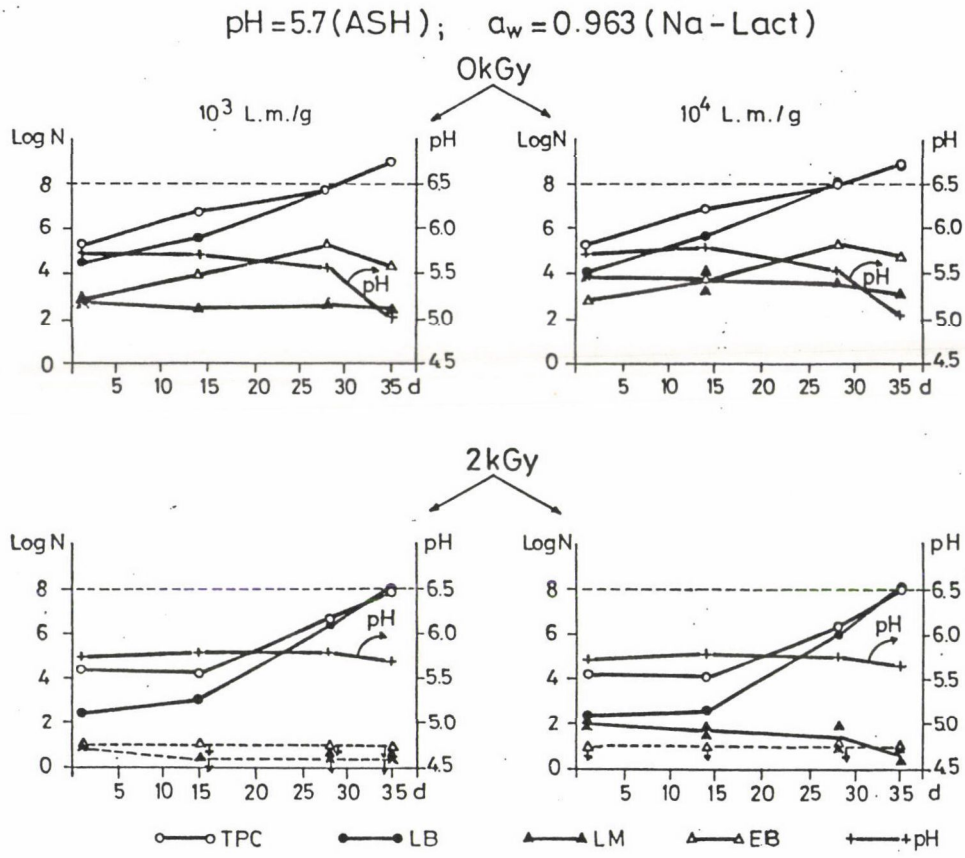

Fig. 1. Survival and growth of the native microflora and the *Listeria monocytogenes* inoculum in untreated and irradiated 'tenderloin rolls' (averages of duplicate estimations at each sampling time) and changes of the pH of the product during 4 weeks of storage at +2 °C following by one additional week of storage at +10 °C. L.m.: *Listeria monocytogenes*; TPC: total aerobic plate count; LB: lactic acid bacteria; EB: Enterobacteriaceae

The results are summarized in Fig. 1. The 2 kGy radiation dose resulted in approx. two log cycles reduction of the cfu-s of *L. monocytogenes* (L.m.), somewhat less in those of lactic acid bacteria (LB), and practically eliminated Enterobacteriaceae (EB) while one log cycle decrease was observed in the total aerobic plate count (TPC). *Listeria monocytogenes* failed to grow even in the unirradiated product, however, it survived well, while its residual populations decreased gradually in the irradiated product during the same post-irradiation storage. For the fourth week of refrigerated storage, lactic acid bacteria became the dominant component of the microflora in all experimental batches.

Conclusions

Combinations of a sensorially acceptable radurization dose and further antimicrobial stress factors may extend not only the shelf-life of specific refrigerated products such as studied by us, but they can improve the microbiological safety, for instance, in case of temperature abuse. The results demonstrate that combining individually ineffective environmental stresses with low-dose irradiation controls efficiently also the growth of *Listeria monocytogenes*. However, it should be noted that a contamination level of *Listeria monocytogenes* of 10^3–10^4 cfu g^{-1} could not be completely inactivated in this minimally processed, extended shelf-life chilled product. Therefore, from the point of view of full microbiological safety, it is important to heat sufficiently this sort of products before consumption to inactivate pathogens eventually present.

*

These studies have been supported by a grant of the National Scientific Research Foundation (OTKA I/3–611) and the Research Contract No. 6271/RB of the International Atomic Energy Agency.

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EFFECT OF IRRADIATION ON THE COLOUR OF GROUND RED PAPRIKA*

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Introduction

Ionizing gamma-radiation may also be applied for red paprika to improve the microbiological quality. However, before the realization of this treatment in practice, it is necessary to investigate the effect of irradiation on the colour of red paprika besides other effects, because the red colour is a very important quality factor (HORVÁTH & KAFFKA, 1973).

Our purposes were to investigate the effects of gamma irradiation with different doses and the storage time on the colour coordinates of the ground red paprika of two types.

Materials and methods

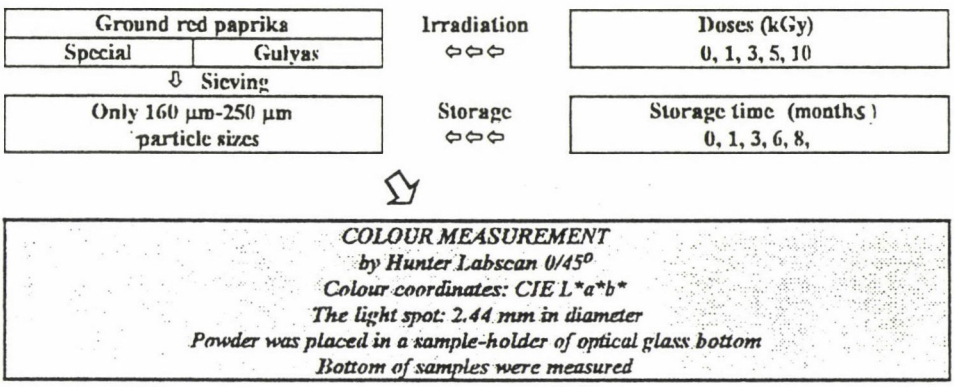

Fig. 1. Blockdiagram of the colour investigation

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Two types of red paprika (Special and Gulyas) one of excellent and one of weak quality (with 5.3 and 2.4 g per kg pigment) were treated and investigated.

The steps of investigation are illustrated in the blockdiagram (Fig. 1).

Discussion

There was no significant difference between the two types of paprika in response to the irradiation and storage. So we discussed only the results of Special type red paprika in this paper.

It can be seen in Fig. 2 that the brightness coordinates (L*) of ground paprika are greater with 1–1.5 units immediately after grinding and irradiation. Red coordinates decrease with only 0.5 units and yellow coordinates (b*) increase with 1–1.5 units in irradiated samples. These changes indicate the discolouration and turning the colour of ground paprika to yellow.

It was found that the degree of the above-mentioned changes were independent of the applied dose, they already occur at 1 kGy. It should be noted that the degree of obtained colour changes are near to or slightly above the border of observable colour differences. They are illustrated with column diagrams in Fig. 3 indicating the border of sensibility. The colour differences were calculated by the CIEL* a* b* formula published by MCLAREN (1981).

Diagrams also show that the change in unirradiated sample exceeds the colour difference with 3.5 units after storage of 8 months. In case of samples irradiated with 3 kGy or greater dose, the change was well observable already after 3 months, showing disadvantageous colour changes of 3 units.

Summary

It was found that radiation treatments with 3 kGy or lower doses caused no considerable colour changes of the ground red paprika during a storage period of 6 months. For samples irradiated with doses higher than 3 kGy the disadvantageous colour changes the decrease of redness were well detectable already after 3 months.

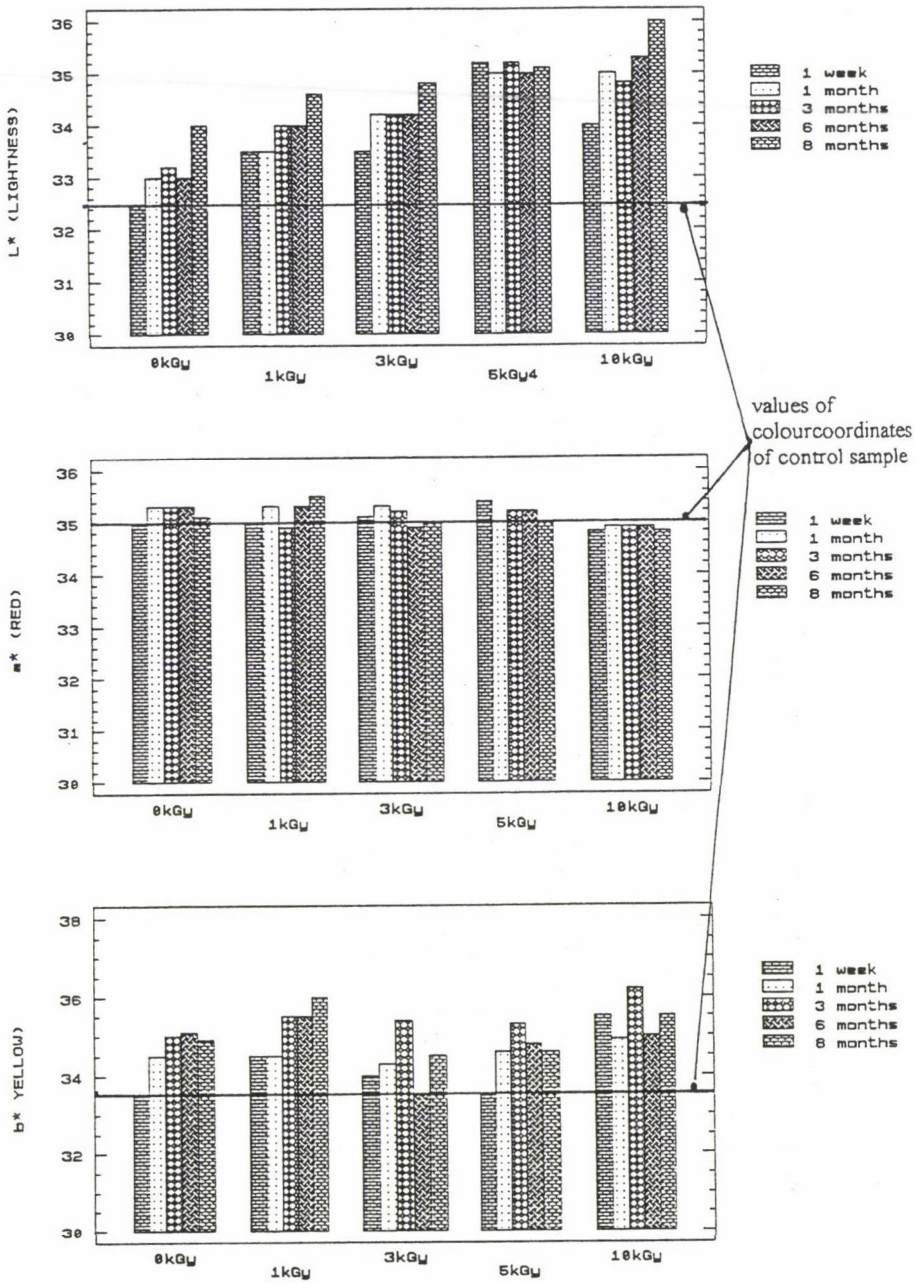

Fig. 2. CIEL*a*b* colour coordinates of control and irradiated red paprika "Special"

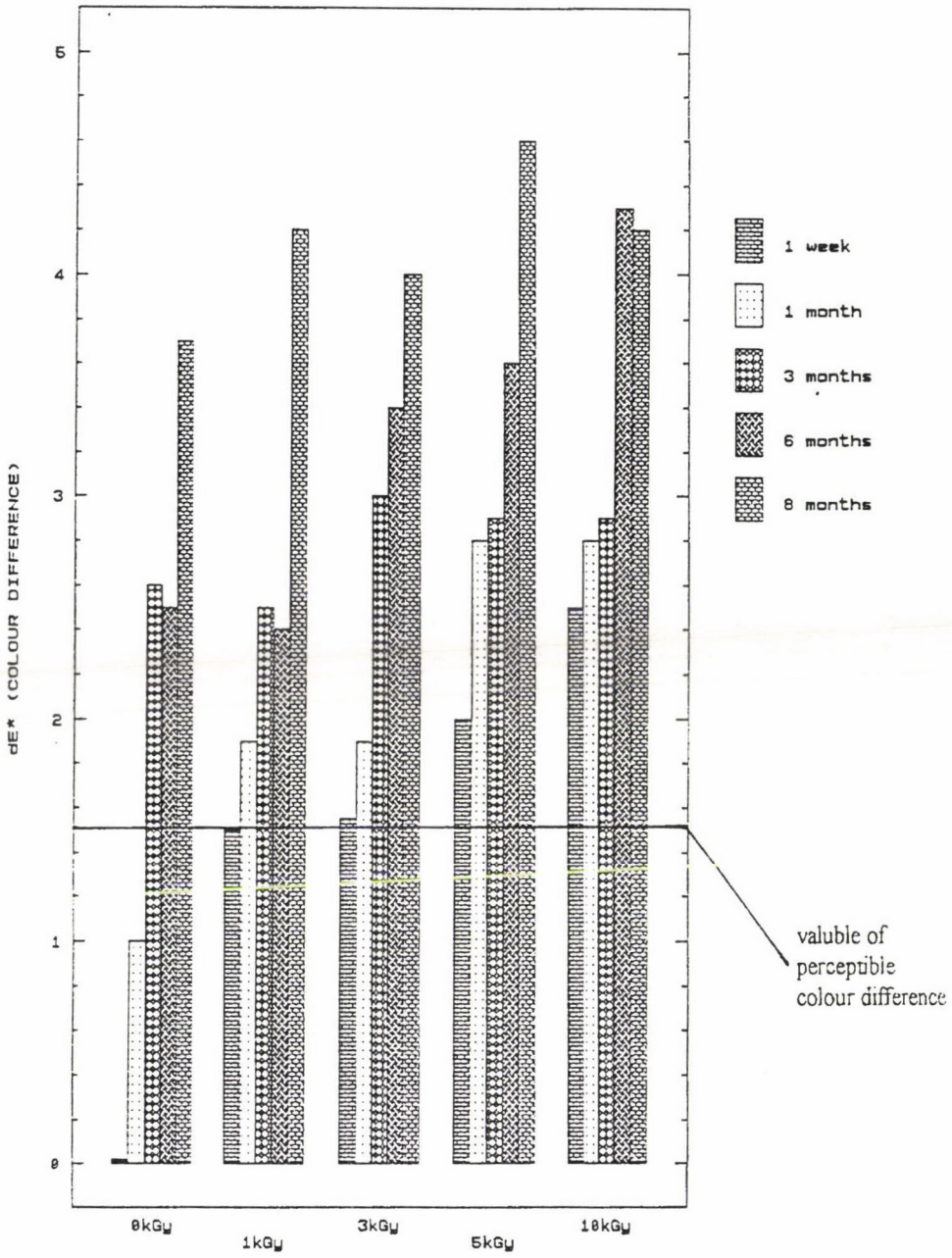

Fig. 3. CIEL*a*b* colour differences of red paprika "Special" (excellent quality) referred them to control sample measured on week 1

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REDUCTION OF VIABLE CELL COUNTS OF HOSPITAL MEALS BY COMBINATION OF GAMMA RADIATION AND OTHER PRESERVATIVE AGENTS*

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Food-borne diseases are serious problems for immuncompromised patients and for small children or old people who have immature or weak immune systems. Immuncompromised patients include those with cancer, human immunodeficiency virus infection, thermal injury, etc. and those receiving immunsuppressive therapy, including bone marrow and organ transplant recipients. The number of these patients is increasing, therefore, there is a need for increasing amounts of more varied meals of low microbial counts. Irradiation and its combinations with other antimicrobial factors reduce very effectively the microbial load of foods and can produce low-microbial and sterile diets. By this way it is possible to improve the nutritional and organoleptic properties of foods as compared to heat sterilized products (HARRISON, 1962; AKER, 1984; IAEA, 1993; SCHMIDL, 1993).

Our objective was to study the effect of a combination of vacuum-packaging, freezing and gamma irradiation on microbiological quality, chemical-, physical- and organoleptical properties of a vegetable plate (green peas) and processed cheeses.

Two hundred g deep-frozen green-peas with margarine addition (5%), as a vegetable plate or component of meals, was vacuum-packed seven layer of PE–PA foil and irradiated with gamma-rays at 10.5–32 kGy dose range in frozen state and stored at –18 °C. The preparation for consumption (defrosting and cooking) was performed by microwave heating and the quality parameters of the cooked product was determined. Three different types of processed cheeses (fat content 45%, thin slices or unsliced) were vacuum-packed in seven layer of PE–PA foil an frozen. The samples were

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irradiated with 12 and 24 kGy at dry ice temperature and stored at −18 °C. After defrosting the samples were analyzed.

Results

The initial microbiological contamination of peas was relatively low. The total aerobic viable cell count (TAVC) of unirradiated samples on the 1st day after irradiation was 2.0 (logN g^{-1}). At the 16 kGy sample the TAVC was less than 1.0. The surviving fraction of microbes decreased during storage in frozen state (Fig. 1). The relative peroxide values of vegetable plate (initial, untreated sample value; 1.0) showed a slow increase, reaching a maximum value of 1.5 in 5 months, after 11 months the unirradiated sample had a value of 1.8 that of irradiated with 32 kGy had 2.2. The hardness (INSTRON 4200) of peas has been reduced as a function of radiation dose. The force at bioyield for 32 kGy treated samples was half of that of untreated peas.

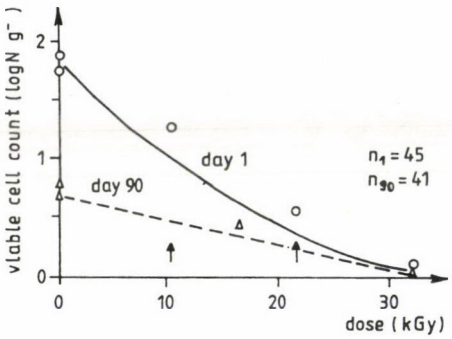

Fig. 1. The total aerobic viable cell counts of "green peas" vegetable plate as a function of irradiation dose and storage time

The panelists could make the difference between unirradiated and irradiated samples by organoleptic testing (triangular test), but average scores of samples were mostly over the acceptable level (3) of the 0–5 score range.

Dairy products are generally sensitive to irradiation. The microbiological contamination of processed cheeses was between the 10^3–10^4 CFU g^{-1} level and they were not very resistant.

The organoleptic properties of cheeses were tested with scoring and triangular test. The panelists (7–9) distinguished the unirradiated and irradiated samples from each other at significant (95% or higher) probability level, but as a function of storage time the number of those panelist preferring irradiated samples was increasing. Evaluation of samples by scoring test, the properties (flavour, odour, texture) were mostly over the acceptable level (Fig. 2).

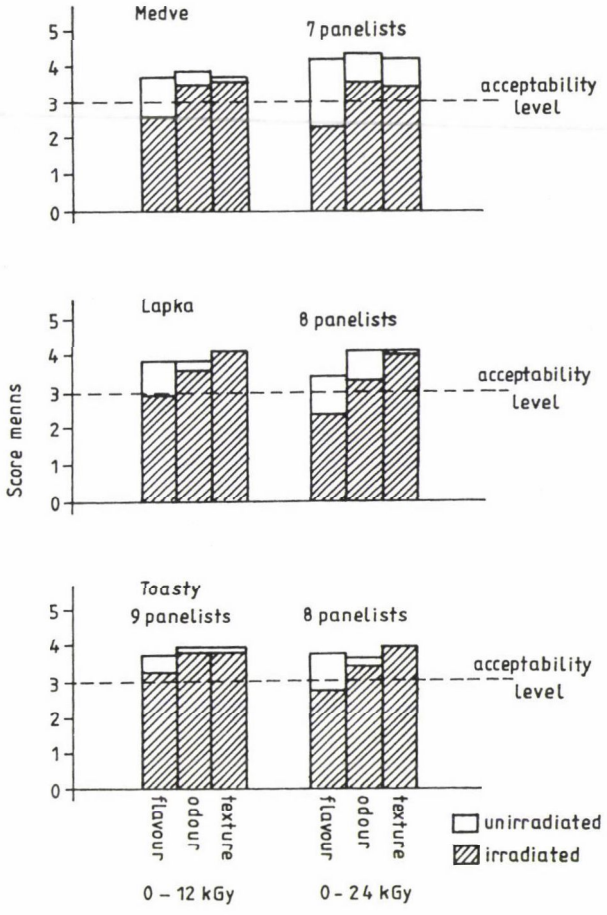

Fig. 2. Scoring test of unirradiated and irradiated processed cheeses on the 1st day after irradiation

Conclusions

Considering the microbiological efficiency of irradiation on frozen vacuum-packed "green peas vegetable-plate", 15–20 kGy dose inactivated the mesophilic aerobic microbes and spores, the surviving fractions were below 10 CFU g^{-1}. The relative PV did not reache the 1.5 during 5 months of freezing storage. Lipid oxidation could be diminished by decreasing the fat content of the "vegetable plate" from 5% to 2%. The excessive softness of peas can be reduced using shorter heat treatment. Organoleptic changes of irradiated peas might be connected with the interaction between peas and packaging material. When the same "vegetable plate" was irradiated in glas jars, no sensorial changes were detectable.

The organoleptic test panel of cheeses showed unwanted changes of flavour and odour which might be related partly to the high fat content, partly to interaction between cheese and the packaging material (plastic foil). Changes of proteins should be considered too. At 12 kGy the changes were smaller than at 24 kGy. It would be advantageous to find more suitable packaging material too.

*

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INFLUENCE OF MINERAL COMPOSITION ON THE THERMOLUMINESCENCE OF IRRADIATED FOODSTUFFS*

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Introduction

Mineral contamination being in or adhering to the foodstuff is generally responsible for the thermoluminescence (TL) phenomenon. Nowadays, mainly measurements on isolated silicate minerals are accepted for the TL method, being described in international protocols, e.g. by CEN (1994).

The aims of this work were to estimate the composition and structure of silicate minerals isolated from paprika powder 'Special', moreover to investigate the radiation-induced properties of their two main components, quartz and feldspar, as functions of absorbed dose and storage time.

Materials and methods

Silicate minerals were separated from paprika powder 'Special' by the isolation procedure described in CEN (1994). During isolation acetic acid and ethanol were also used instead of the proposed hydrochloric acid and acetone, since less damage was revealed in the crystal structure.

Potassium feldspar microcline ($KAlSi_3O_8$) and crystalline quartz as probable mineral components were investigated. Grain size was 40–60 μm for both minerals.

The samples were irradiated with 1, 2.5, 5 and 10 kGy doses by means of a ^{60}Co-radiation source. Radiation-induced TL signals of the two minerals were followed for 6 months.

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Structure and composition studies were carried out with an X-ray diffractometer type DRON-UM-1 (Buravesztnyik, St Petersburg, Russia) and an X-ray analyser type EMG-8500 NZA-416 (EMG, Budapest, Hungary), respectively. TL glow curves were recorded with a linear heating rate of 6 K/s, using a Harshaw 4000A TLD reader (Solon, Ohio, USA).

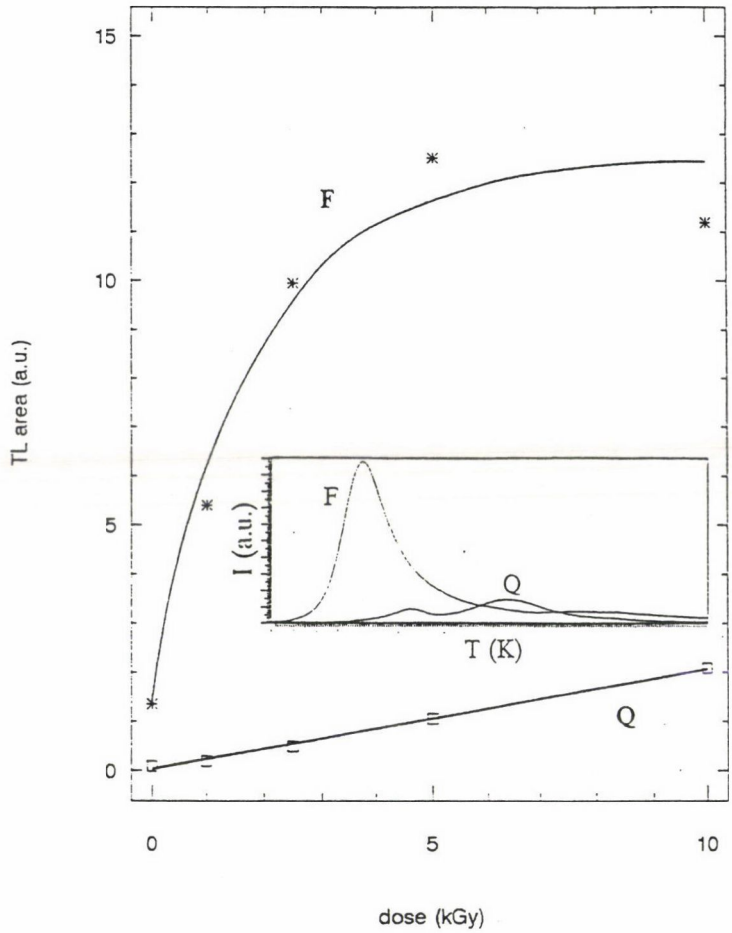

Fig. 1. Areas under the TL curves as a function of absorbed dose after 1 month for potassium feldspar (F) and crystalline quartz (Q). Insert: TL curves of F and Q after irradiation treatment with 5 kGy. (a.u.: arbitrary unit)

Results and discussion

After both isolation procedures, quartz and feldspar could be identified in the diffraction spectra of the mineral fraction isolated from paprika powder 'Special'. These results confirm those of PINNIOJA and co-workers (1993) and CALDERÓN and co-workers (1995) who also used X-ray diffraction for mineral analyses. Using acetic acid and ethanol, the peak intensities of quartz and feldspar are considerably greater than applying the usual hydrochloric acid and acetone.

It was found that tungsten remained from the sodium polytungstate solution used in isolation procedure, and its content was higher using hydrochloric acid and acetone. Significant composition changes were found only for two elements: the concentrations of Si and Ca were 20% and 0.88% using hydrochloric acid and acetone, while they were 33% and 0.59% using acetic acid and ethanol, respectively. The elemental composition of the dust on spices has been studied by GÖKSU and co-workers (1990) who employed proton induced X-ray emission (PIXE).

Dose dependence of the area under the TL glow curves for potassium feldspar (F) and quartz (Q) is given in Fig. 1. TL glow curves of these minerals are shown in the insert of Fig. 1; peak temperature is about 430 K for F, while they were about 480

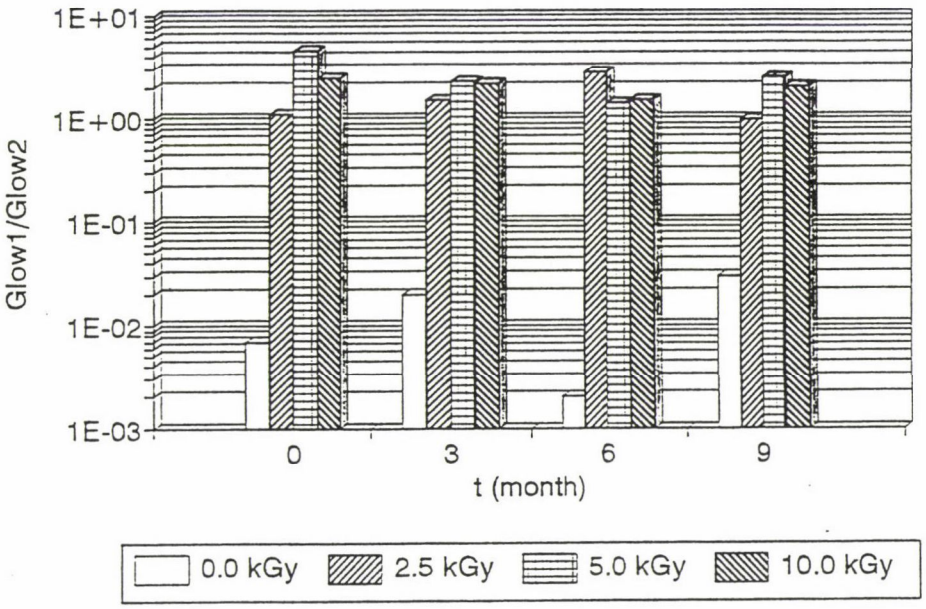

Fig. 2. TL glow ratios of potassium feldspar as functions of storage time and absorbed dose

and 580 K for Q. It was found that TL sensitivity of F is considerably better at lower doses but it has a saturation tendency, and that of Q is rather low but linear in the investigated dose interval.

Ratios of the areas under TL curves (Glow 1/Glow 2) as functions of storage time and absorbed dose are presented in Fig. 2 for feldspar. (Glow 1 and 2 mean the TL intensity integrated in total temperature interval, i.e. the area under the TL curve, before, and after re-irradiation with 1 kGy (CEN, 1994). This ratio is sensitive enough for detecting irradiation, since in the case of an irradiated sample, it can be greater by several orders of magnitude than that of an unirradiated one. (Note that in every case the ratios were less for quartz.)

Conclusions

In order to demonstrate an irradiation treatment, the application of acetic acid and ethanol in the isolation procedure seems to be more suitable than that of hydrochloric acid and acetone: the crystal structure of the mineral fraction is less damaged, and more intensive TL signal is detected.

Concerning the TL response, among the components of silicate minerals, feldspar may be the dominant part responsible for TL, but TL sensitivity may depend on the type of the feldspar (SANDERSON, 1990).

*

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IS IT POSSIBLE TO DETECT THE IRRADIATION TREATMENT OF HUNGARIAN PAPRIKA AFTER LONG-TERM STORAGE?*

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Introduction

Irradiation offers an efficient means of decontaminating spices while simultaneously retaining most of their valuable sensory quality; in 1992, more than 20 000 t of spices were irradiated worldwide (LOAHARANU, 1994). In international trade it would be desirable to have analytical detection methods to identify an irradiation treatment on the product itself, in addition to the documentary records from the radiation facility, accompanying the product (DELINCÉE, 1993). Several analytical methods have been proposed for spices, among them thermoluminescence (TL).

The aim of this work was to investigate the detectability of irradiation of Hungarian paprika powders by TL method. The measurements were made on two whole paprika powders ('Special' and 'Goulash'), on fractions of different grain sizes of these powders and on minerals separated from the powders as functions of absorbed gamma-dose and storage time.

Materials and methods

Whole paprika powders – produced at the Paprika Co. (Szeged, Hungary) – were selected into 5 fractions of different grain sizes (0–160, 160–250, 250–315, 315–400 and 400–500 μm). Silicate minerals were isolated from the whole samples with a procedure described by CEN (1994).

Samples were treated with 2.5, 5 and 10 kGy doses by means of a ^{60}Co-radiation source. Storage measurements were done for 9 months.

* Extended abstract of a poster presented at the Symposium on Current Aspects of Food Irradiation held in the frame of IUFoST 9th Congress of Food Science and Technology, 3 August 1995, Budapest, Hungary

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TL of whole and fractionated samples was measured in pure nitrogen atmosphere with a linear heating rate 4.5 K s^{-1} in temperature interval 300–600 K by means of a TL dosimeter type NHZ-203 (Central Research Institute for Physics, Budapest, Hungary). The area under the TL curve, while the difference between TL areas of irradiated and unirradiated samples – as characteristic data – were designed A and ΔA, respectively.

TL measurements on isolated minerals were carried out with a TL reader type ELSEC 7185 (Littlemore, Oxford, England), the measuring chamber being flushed with pure nitrogen. The heating rate was 6 K s^{-1}, using a heating time of about 70 seconds depending on the final temperature of 673–773 K. According to the CEN draft (1994), samples were re-irradiated with 1 kGy after the first recording (Glow 1) and were measured again (Glow 2).

Results and discussion

Since the results of the two paprika powders were basically similar, those obtained with paprika 'Special' are reported here.

The time dependence of ΔA values for whole samples of paprika 'Special' is represented in curve 1 of Fig. 1; this value decreased to 0 within 28 days in an exponential-like decay.

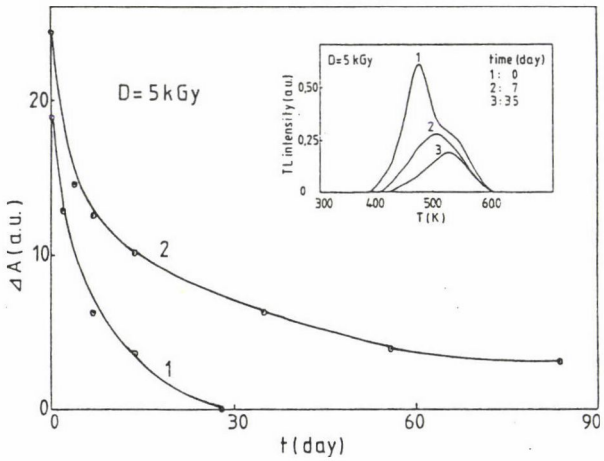

Fig. 1. Time dependence of ΔA values of paprika powder 'Special' measured on (1) whole samples and (2) fractionated samples of grain sizes below 250 μm. Insert: TL glow curves of paprika fraction samples with grains below 250 μm at different times. (a.u.: arbitrary unit)

It was found for the TL data of irradiated (5 kGy) and unirradiated paprika fractions that ΔA values decreased with increasing grain size, indicating that the components responsible for TL, namely the contaminating silicate minerals, mainly exist in grains of lower sizes. This assumption is confirmed by curve 2 of Fig. 1, which represents data obtained on samples of grain sizes below 250 μm, and ΔA remained significant even after 80 days, offering a possibility for the unambiguous detection of radiation treatment.

The TL curves of fractions of grain sizes below 250 μm exhibit typical changes during storage (the insert of Fig. 1). On the basis of this phenomenon, a quick and rapid screening measurement could be suggested for detecting irradiation of paprika powders still after storage of several months, using the fraction of grains below 250 μm after sieving.

The TL glow ratios for isolated minerals as a function of storage time are illustrated in Fig. 2. It was found that the radiation treatment could be unambiguously detected even after 9 months at each applied dose. Furthermore, the ratios for irradiated and unirradiated samples are different by a magnitude of several orders, independently of grain size as expected. (In every case, the irradiated samples showed higher TL glow ratios than 0.5, while the unirradiated ones' values were below 0.1.)

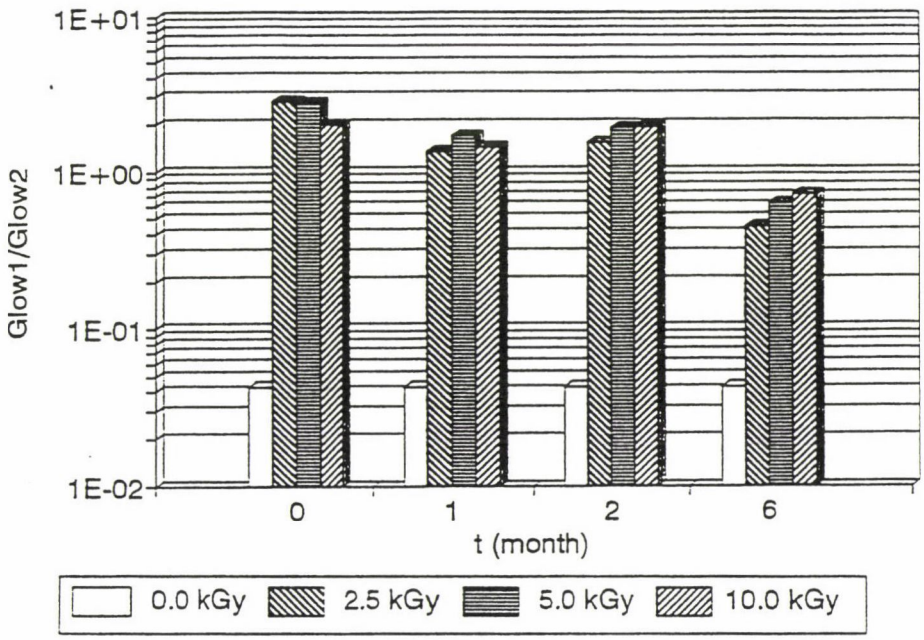

Fig. 2. TL glow ratios of minerals isolated from whole paprika powder 'Special' as functions of absorbed dose and storage time. Integration interval: 443–519 K

Conclusions

Thermoluminescence measurements are suitable for the detection of irradiation treatment of paprika powders. If fractions of powders with grain sizes below 250 μm are used for the TL measurements, a rapid screening may be performed to identify irradiated items within three months of storage. Unequivocal identification of irradiated paprika powders, however, is obtained by the more laborious procedure of isolating silicate minerals from the paprika samples, and determining the TL glow ratios of these minerals. This procedure allows the detection of irradiated paprika even after long-term storage, checked in this paper for a period of nine months, but also longer stored irradiated paprika would most probably be identified as so treated.

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STUDY ON OXIDIZED CHOLESTEROL DERIVATIVES IN FOODSTUFFS*

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Introduction

Cholesterol can easily be oxidized in the presence of air. Light, high temperature, ionising radiation, free radicals promote the formation of numerous cholesterol oxidation products (COPs). Some of these derivatives are considered as a more potent risk factors in the progression of coronary heart disease and atherosclerosis than cholesterol itself, furthermore they are cytotoxic, mutagenic and influence the cholesterol biosynthesis (PENG & MORIN, 1992). The aim of this work was to study some of hardly known parameters which might promote the cholesterol oxidation, among others: high temperature, microwave treatment, ionising radiation, long term storage. The influence of packaging conditions and antioxidants on the suppression of oxysterols formation were also examined.

Methods

Egg powder, pastry products, fat, minced chicken meat were used in different experiments. Following lipid extraction by Folch's method and hot saponification in nitrogen atmosphere was employed for separation of sterols. The non-saponifiable lipid fraction containing cholesterol and its derivatives was separated by TLC. The quantities of oxysterol compounds in separated and eluted spots of silica absorbent were measured by adapting of enzymatic determination of cholesterol based on the reaction of cholesterol oxidase (RICHMOND, 1973) which can oxidize not only cholesterol, but also its oxidized derivatives.

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Results and discussion

In previous reports TLC was used for the separation and identification of oxysterols in gamma-irradiated egg powder (LEBOVICS et al., 1992, 1994). Levels were usefully estimated by visual comparison of spots with those of authentic standards. The combination of TLC and enzymatic method is advantageous for a more precise quantification of some cholesterol oxides even in the presence of large quantity of cholesterol. The predominant products formed during irradiation and other treatments were 7α-hydroxycholesterol, 7β-hydroxycholesterol (these are early and sensitive indicators of oxidative changes of cholesterol), but cholesterol-5α,6α-epoxide, 7-ketocholesterol, cholestan-3β,5α,6β-triol, 25-hydroxycholesterol were also detectable. Formation of oxysterols were accelerated by gamma-irradiation when 1, 2, 4, 6 kGy was used for inactivation of Salmonella in egg powder.

Dried whole egg, irradiated under aerobic conditions in polyethylene package contained 40 mg kg^{-1} 7-hydroxycholesterol isomers (7α and β), but vacuum-package (laminated aluminium foil covered with polyethylene outside and polyester inside) considerably suppressed the formation of these compounds (7 mg kg^{-1}). Either in freshly produced control minced chicken meat, or in those irradiated with 2, 4, 6 kGy, the levels of 7-hydroxycholesterol derivatives did not exceed 1 mg kg^{-1}, but during 12 months storage at –18 °C of chicken meat, treated with 4 kGy, 48 mg kg^{-1} 7-hydroxycholesterol isomers and 10 mg kg^{-1} 7-ketocholesterol were measured. Table 1 shows the quantities of 7-hydroxycholesterol isomers and 7-ketocholesterol forming in the irradiated minced chicken-meat, in the presence of antioxidants during 12 months storage. The concentrations of alpha-tocopherol and BHT added to the meat samples were in the range of officially allowed quantities. Data show, that BHT proved more effective in prevention of further oxidation, than alpha-tocopherol. In fresh sponge cake, baked in gas-oven, cholestan-3β,5α,6β-triol were detected due to the high temperature. Dry pastry products purchased in local shops contained oxysterols in the range of 1–8 mg kg^{-1}. Microwave treatment of fat during 5 min (152 °C) resulted in formation of 7-hydroxycholesterol isomers, 7-ketocholesterol and cholesterol-5α, 6α-epoxides only in traces. After 15 min (217 °C) the levels of COPs moderately increased.

More work seems necessary to follow up the undesirable chemical changes of cholesterol considering the technological steps during industrial and home processing of foodstuffs.

Table 1

Oxidized cholesterol levels in gamma-irradiated frozen minced chicken meat after 12 months storage at -18 °C in the presence of antioxidants[a]

Dosage levels (kGy)	Oxysterols	BHT (mg per 100 g)			alpha-tocopherol (mg per 100 g)		
		0	20	40	0	12	20
2	7α-hydroxycholesterol	6.0	4.2	4.0	6.0	6.0	4.9
	7β-hydroxycholesterol	5.9	3.7	3.8	5.9	5.4	4.0
	7-ketocholesterol	4.5	nd	nd	4.5	2.1	2.9
4	7α-hydroxycholesterol	23.0	6.8	3.2	23.0	11.2	8.5
	7β-hydroxycholesterol	25.0	6.5	2.7	25.0	13.4	9.0
	7-ketocholesterol	10.0	4.1	3.9	10.0	6.1	6.1

[a] Data of duplicated samples in two parallel measurements are given in mg kg^{-1}
cholesterol: 1050 mg kg^{-1}
nd: <0.4 mg kg^{-1}

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EFFECTS OF COMBINED TREATMENTS ON SPORE FORMING BACTERIA – POTENTIALITIES OF THE MALTHUS INSTRUMENT*

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(Received: 31 January 1996; accepted: 7 February 1996)

Introduction

For establishing a growth curve plate count is the most reliable method, but it is very time and material consuming. It is also possible to monitor bacterial growth by recording the change in electrical conductance of the growth medium (RICHARDS et al., 1978; BAYNES et al., 1983). However it has to be taken into consideration that only the last part of the curve is measured and in some cases it makes this method unreliable (CUPPERS & SMELT, 1993).

This communication would like to draw attention to possible errors occurring when a Malthus method is applied, how it can be turned into advantage and utilized in the research of the effects of combined treatments.

Materials and methods

The measurements were carried out with *Bacillus cereus T* aerobic mesophilic spore forming bacteria. Spores were obtained by spreading the suspension over plates of PCA. Harvested spores were washed in distilled water by centrifugation, stored in phosphate buffer at 4 °C.

For the heat treatment 4 ml screw-capped glass tubes were filled with 10^6 cm^{-3} spore suspension. The treatment was carried out in 90 °C water bath then immediately cooled in ice water.

The pH of the growth medium was adjusted to 5.9 with 1 N HCl.

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The spore suspension was irradiated in a RH-γ-30 Co^{60} gamma source in 0–6 kGy dose range.

The survival was determined by plating the treated suspension in PCA pH=5.9. The plates were incubated at 37 °C for 1–7 days.

The outgrowth of spores was monitored with Malthus conductance meter. From the suspensions 0.2 ml was transferred into the cell containing 2.8 ml Malthus Columbia Broth medium. The cells were incubated at 37 °C and the conductance was monitored continuously.

Results and discussion

1. Principles of the Malthus measurement

In a Malthus measurement the received results are evaluated with the help of a calibration curve. Generally the suspensions of different initial counts are prepared from dilution series, and the initial cell counts obtained by plating methods are plotted against the measured detection times (Fig. 1). Thus the characteristics (growth rate, lag-time) of the microbes present in each sample are the same as a result of the same origin.

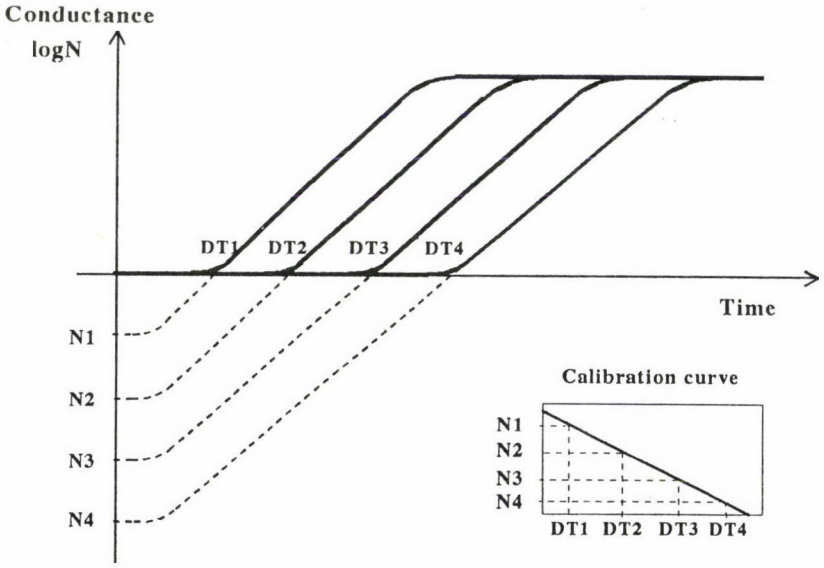

Fig. 1. The theory of Malthus measurement and the obtaining of a calibration curve. N_{1-4} are the logarithms of the initial cell counts and DT_{1-4} are the measured detection times

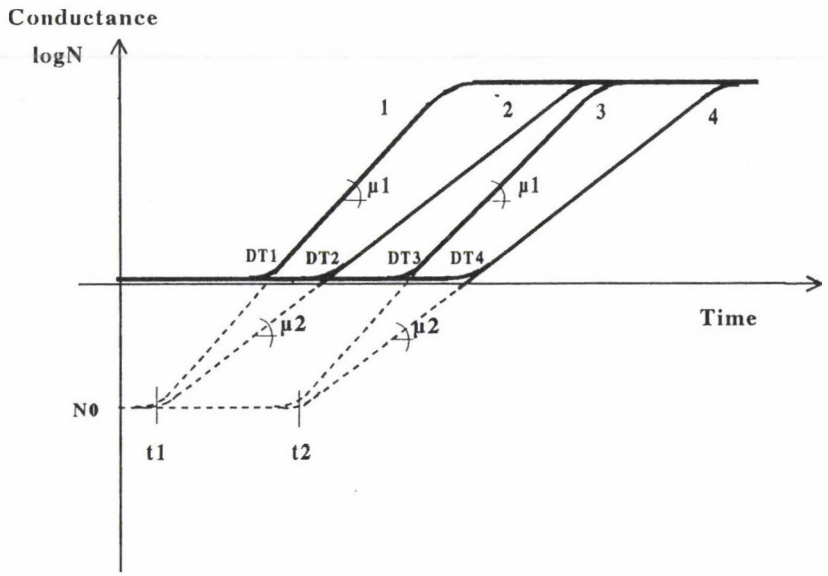

Fig. 2. Possible errors occurring during the Malthus measurement due to the high detection limit (approx. 10^6–10^7 microbes cm^{-3})

2. *Possible errors*

However, there is no guarantee that the microbes in an unknown sample would behave as the ones used for making the calibration curve. And if the growth rate (2), the lag-time (3) or both (4) differ from the standard (1), highly different detection times are obtained from the same initial count (Fig. 2). In a case like this the difference between the calculated result and reality can be as big as several orders of magnitude.

3. *Appearance in practice*

In Fig. 3 the percentage survival of irradiated *Bacillus cereus* T spores is shown. The straight line represents the percent of the surviving spores received by plating method, the scattered line shows the percent of survivors obtained by the measurement with the Malthus instrument. Since a calibration curve prepared with uninjured bacteria is used for the calculations of the initial count from the measured detection times, the results are the theoretical initial numbers of uninjured bacteria that are needed to give the obtained detection times. As lower numbers of uninjured microbes are necessary to give the same detection times as injured bacteria, the two curves will differ from each other. The difference indicates the injury of the spores due to the treatment.

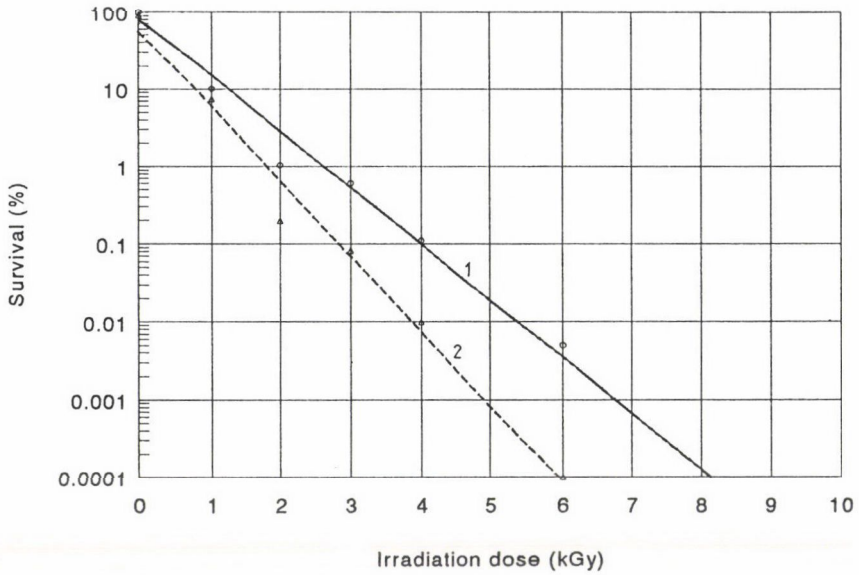

Fig. 3. The effect of irradiation on the survival of *Bacillus cereus* T spores determined by plating method (1) and Malthus measurement (2)

4. Utilization

When the effect of a combined treatment like heating and then plating in acidified medium is to be investigated, different calibration curves can be used: a calibration curve with uninjured cells, one with heat treated cells and another with the pH inhibited cells. If the measured detection times are evaluated with each calibration curves three different survival curves, i.e. three different initial cell counts are obtained. In Fig. 4 these curves are shown. Curve 1 represents the real survival of the treated spore suspension (obtained by plating method), Curve 2 is the result calculated from the detection times according to the calibration curve with uninjured cells. The difference shows the degree of injury. Curve 3 represents the rate of inhibition calculated with the calibration of the pH treated cells. Its difference from the real survival shows the degree of injury caused by the other agent (in this case the heating). Curve 4 is obtained by the calculation based on the calibration with heated cells, its difference from the real survival shows the degree of inhibition caused by the acidified medium.

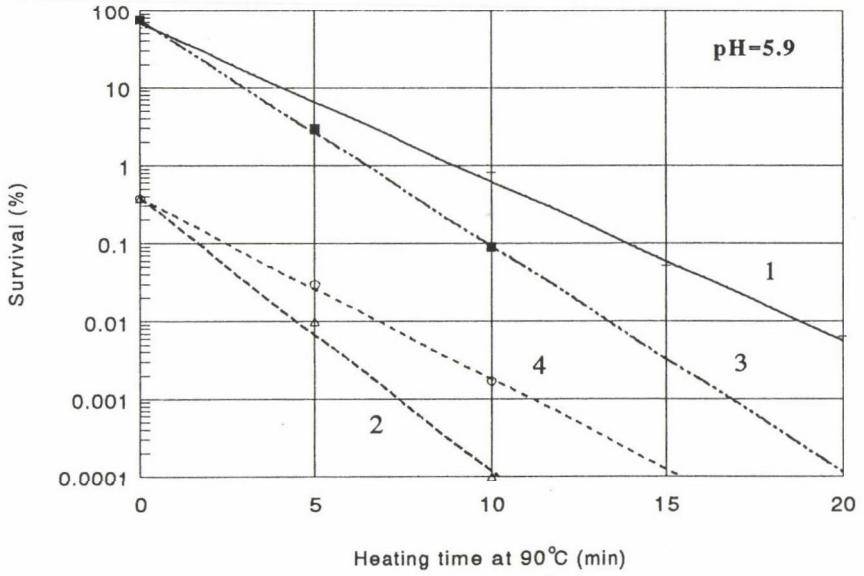

Fig. 4. The combined effect of heating and the pH of the recovery medium on the survival of *Bacillus cereus* T spores determined by plating method (1) and Malthus measurement calibrated to untreated cells (2), heat treated cells (3) and untreated cells grown in recovery media of different pH (4)

Conclusions

– In Malthus measurement good calibration curve is essential for obtaining real initial counts. As the prelife of the food is not known in most of the cases, the injury to the microbes caused by the previous treatments can not be compensated with calibration curves, so the results obtained should be handled with caution.

– The Malthus measurement can be very useful in the study of combined treatments. With different calibration graphs the effects of the different agents can be studied separately.

*

The measurements were carried out in the RIKILT-DLO, Wageningen, The Netherlands, under the guidance of Mr. H. STEGEMAN.

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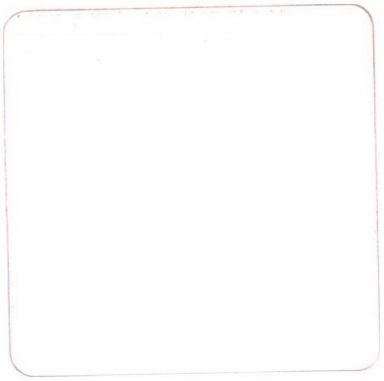

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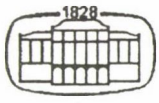

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BUDAPEST

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EVALUATION OF COLOUR VALUE AND PIGMENT CONCENTRATION OF CAPSICUM EXTRACTS

K. V. BALAKRISHNAN[a,c], J. VERGHESE[a] and D. JOSEPH FRANCIS[b]

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This study on capsicum extracts is designed: (1) to evaluate the response of the major red and yellow pigments to the colour value; (2) to investigate the accuracy of spectrophotometric methods for the estimation of the red, yellow and total pigments; (3) to confirm the trustworthiness of commonly adopted conversions of colour value to pigment concentration and (4) to re-examine the existence of the alleged equilibrium between the red, yellow and total pigments.

Firstly, it is shown that the colour value as measured by the classical EOA, ASTA or MSD-10 methods cannot be accepted as a quality determinant of capsicum extracts. Secondly, the spectrophotometric methods of BENEDEK, FEKETE & co-workers and HASPEL-HORATOVIC & HORICKOVA (1976) fail to quantify the red, yellow and total pigments accurately. Thirdly, no equilibrium exists between the red, yellow and total pigment concentrations. However, the MSD-10 colour value divided by 1600 or the ASTA colour value by 40 approaches the total pigment concentration as disclosed by HPLC method. To quantify the red and yellow pigments, HPLC method is *par excellence*.

Keywords: capsicum extracts, colour value, red and yellow pigment concentrations

Colour is a prized property of carotenoid concentrates derived from capsicum. This property is essentially the cumulative contribution of the red and yellow pigments in the spice (SHUSTER & LOCKHART, 1954; SZABO, 1970; SALZER, 1977). The red pigments constitute about 70 to 85% and yellow, about 15 to 23% of the pigment pool (SZABO, 1970; SALZER, 1977; GOVINDARAJAN et al., 1987). Interest lies in the red components and others play only a subordinate role (MOSTER & PRATER, 1952; SHUSTER & LOCKHART, 1954; PURSEGLOVE et al., 1987).

The most popular quality determinant of capsicum extract for food colouring is its colour value (CV) (MENDEZ et al., 1993). Measurement of this factor by EOA

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(1965), ASTA (1985) or MSD (1959) method exploits the absorbance of a dilute acetone solution of the colour matter at 458, 460 and 462 nm respectively; the optical density obtained is multiplied by appropriate constant to give the CV. But how the individual colour components in capsicum concentrates influence the colour value is not known.

Further, for evaluating the capsicum extracts, a knowledge of red, yellow and total pigment concentration in them is of importance. Utilising open column (CURL, 1962; DE LA MAR & FRANCIS, 1969), thin layer (TLC) (VINKLER & RICHTER, 1972) and high pressure liquid (HPLC) (BARANYAI et al., 1982; GREGORY et al., 1987; PHILIP & CHEN, 1988; MEJIA et al., 1988; BIACS et al., 1989) chromatography and spectrophotometric techniques (MOSTER & PRATER, 1952, 1957; BENEDEK, 1958; POHLE & GREGORY, 1960; ANDRE, 1973; FEKETE et al., 1976a, 1976b; HASPEL & HORICKOVA, 1976; WOODBURY, 1977; MALCHEV et al., 1982; VARGA et al., 1984), this problem has been examined in depth. Of these, the simple and rapid methods of BENEDEK (1958), FEKETE and co-workers (1976a,b) and HASPEL-HORATOVIC and HORICKOVA (1976) do not give agreeable results.

It is reported (VINKLER & RICHTER, 1972) that there exists an equilibrium between the red, yellow and total pigment content in the spice, ie. the percentage of red components increases with increase in total pigment content and that of yellow components decreases correspondingly.

A simple correlation suggested (SZABO, 1970) to arrive at pigment concentration is that pigment content in g/kg obtained according to BENEDEK's (1958) method is equivalent to EOA colour value divided by 1600. Another approach is that 40 ASTA colour units approximates a pigment concentration of 1 g/kg; however, this conversion factor has not been backed by published data.

For quick assessment of capsicum extract, the ratio of absorbance values in acetone at 472 and 454 nm is also recommended as a yardstick (MAYER, 1989), but no framework is available as to the limits of this ratio to compositional variation of red–yellow pigment system of the concentrates.

The colour strength of capsicum extract has also been evaluated in terms of micrograms β-carotene per gram of the sample (POHLE & GREGORY, 1960; TANDON et al., 1964; DE LA MAR & FRANCIS, 1969; MATHEW et al., 1971; RAMAKRISHNAN & FRANCIS, 1973; GOPALAKRISHNAN et al., 1987). This determination is based on the assumption that β-carotene has colour characteristics very similar to the colouring matter of capsicum. How β-carotene can represent the complex mixture of pigment components in capsicum extract is not quite clear.

Our objectives were to evaluate the response of the major red and yellow pigment components to the CV; to investigate the reliability of the three simple and

rapid spectrophotometric methods in estimating the red, yellow and total pigment concentration; to confirm the trustworthiness of the commonly used conversions of CV to pigment concentration and to re-examine the existence of an equilibrium between the red, yellow and total pigment content in capsicum extracts.

1. Materials and methods

1.1. Materials

β-carotene, purity > 97% (Fluka) and pure capsanthin and lutein (gifts from Laboratorios Bioquimex, S. A. DE C. V., Mexico) were used. Other carotenoid components for co-HPLC were isolates from capsicum extract by TLC. Analytical grade reagents were used for spectrophotometry and TLC, and chromatography grade for HPLC. Capsicum extracts of varying colour strengths were Synthite's products. Capsicum extract of approx. 40,000 colour units is designated as C–40.

1.2. Methods

CVs were determined by EOA, ASTA and MSD–10 and quantitative estimation of pigments by spectrophotometric methods (BENEDEK, 1958; FEKETE et al., 1976a, 1976b; HASPEL-HORATOVIC & HORICKOVA, 1976) in a Hitachi U–2000 Spectrophotometer. TLC runs were carried out as per procedure earlier reported (VINKLER & RICHTER, 1972). HPLC analyses were carried out on saponified samples in a Hewlett-Packard Series 1050 High Pressure Liquid Chromatrograph using two Supelcosil LC–SI (3 micron) columns, 15.0 cm × 4.6 mm ID and 7.5 cm × 4.6 mm ID connected in series; n-hexane – ethyl acetate (65:35) was used as mobile phase.

2. Results

CVs of β-carotene, C–40 and C–40 fortified with 1, 2 and 3% β-carotene obtained by EOA, ASTA and MSD–10 methods are recorded in runs 1, 2, 3, 4 and 5, respectively of Table 1. Results of similar runs by addition of capsanthin are listed in runs 6–9.

β-carotene and capsanthin display high CV of 15,13,380 and 15,57,600 MSD–10 colour units respectively, approximately 35–36 times that of C–40 concentrate. With increments in these pigment constituents, the CV of capsicum extract increases; C–40 doped with 3% β-carotene or capsanthin experiences doubling of CV of the original. Clearly then, CV of capsicum extract leans on the colour value of the pigment components. Thus, a high CV of capsicum extract is no guarantee to richness in red pigments.

Table 1

Colour value of C-40 dosed with varying percentages of β-carotene and capsanthin

Run No.	Substrate	Method					
		EOA		ASTA		MSD-10	
1	2	$\bar{x}$ 3	±s 4	$\bar{x}$ 5	±s 6	$\bar{x}$ 7	±s 8
1	C-40	39 995	122	1 069	7	42 768	132
2	β-Carotene	1 480 470	5 060	38 950	259	1 513 380	4 620
3	C-40 (99%) + β-carotene (1%)	55 144	183	1 466	8	58 146	198
4	C-40 (98%) + β-carotene (2%)	73 261	245	1 931	12	76 758	264
5	C-40 (97%) + β-carotene (3%)	83 448	283	2 204	16	87 252	271
6	Capsanthin	1 415 200	6 100	37 720	328	1 557 600	5 640
7	C-40 (99%) + capsanthin (1%)	54 290	244	1 459	11	58905	187
8	C-40 (98%) + capsanthin (2%)	68 015	198	1 824	9	74 250	215
9	C-40 (97%) + capsanthin (3%)	81 282	311	2 205	13	89 265	245

$\bar{x}$: mean value of 4 measurements

±s: standard deviation of 4 measurements

Table 2

Pigment distribution by different spectrophotometric methods, g/kg

Run No.	Substrate	Method											
		BENEDEK		FEKETE & co-workers					HASPEL & HORICKOVA				
		Total pigments		Red pigments		Yellow pigments		Total pigments	Red pigments		Yellow pigments		Total pigments
		$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s		$\bar{x}$	±s	$\bar{x}$	±s	
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	C-40	21.97	0.37	14.30	0.31	12.25	0.24	26.55	15.80	0.27	27.57	0.35	43.37
2	C-40 (99%) + β-carotene (1%)	31.69	0.46	21.15	0.51	15.60	0.33	36.75	21.13	0.40	36.44	0.45	57.57
3	C-40 (98%) + β-carotene (2%)	40.56	0.39	27.74	0.44	18.08	0.31	45.82	25.30	0.28	42.40	0.41	67.70
4	C-40 (97%) + β-carotene (3%)	49.99	0.62	33.82	0.55	22.04	0.42	55.86	27.23	0.33	50.40	0.51	77.63
5	C-40 (99%) + capsanthin (1%)	30.59	0.47	27.70	0.31	10.23	0.22	37.93	27.12	0.50	23.33	0.54	50.45
6	C-40 (98%) + capsanthin (2%)	40.49	0.38	40.59	0.46	7.69	0.30	48.28	39.72	0.33	14.34	0.29	54.06
7	C-40 (97%) + capsanthin (3%)	49.33	0.58	53.01	0.75	4.79	0.35	57.80	51.99	0.61	7.12	0.42	59.11

$\bar{x}$: mean value of 4 measurements

±s: standard deviation of 4 measurements

Table 2 summarises the pigment concentrations obtained by the different spectrophotometric methods. Column 3 gives the total pigment content by BENEDEK's (1958) procedure, without shedding any light on the quantum of red and yellow principles, either individually or collectively. Each 1% increment in β-carotene/ /capsanthin is reflected by a corresponding projection of ca 10 g/kg to the pigment pool. Thus, BENEDEK's (1958) method is sensitive and is useful for rapid objective measurement of total pigments in capsicum extracts.

Columns 5, 7 and 9 of Table 2 display the distribution of red, yellow and total pigments by the method of FEKETE & co-workers (1976a,b). As compared to the value obtained by BENEDEK's method, here the total colour matter is distinctly higher. What is puzzling, however, is the twist in the distribution pattern of red and yellow pigment groups. β-carotene resurrects partly in the red and partly in the yellow pigment pool, though the total hike of ca 10 g/kg for 1% increment is almost in harmony with the expected theoretical equivalent. For each 1% increment in capsanthin, the red pigment concentration increases by 13 g/kg, ie., 3 g/kg in excess of the theoretical figure. However, the yellow pigments show a fall by ca 3 g/kg, keeping the net hike at ca 10 g/kg. Hence, it is concluded that this method is unlikely to ensure correct quantification of red and yellow pigments.

Finally, in columns 10, 12 and 14 of Table 2 are assembled the red, yellow and total pigment concentrations as determined by the method of HASPEL-HORATOVIC & HORICKOVA (1976). The estimated total pigment content is higher than that by the other methods. As in the above case, β-carotene is partitioned between red and yellow pigments. For incremental addition of 1% capsanthin, the red pigments increase by ca 12 g/kg; on the other hand, the yellow pigments diminish by 4–9 g/kg so that the net hike in the total pigment content is less than the calculated value.

In Table 3 are assembled the CV and HPLC distribution of pigments in 8 samples of capsicum extracts. Samples 1–8 display colour values which increase in that order. Clearly then, there is no correlation between the percentage of red or yellow components and the total pigment concentration; this conclusion is contrary to that of early workers (VINKLER & RICHTER, 1972). The results of Table 1 and Table 2 also back the above conclusion.

Table 3 also gives the EOA CV/1600 value and the corresponding pigment concentration by BENEDEK's (1958) procedure for the 8 samples. The EOA CV/1600 value is higher than the pigment content through BENEDEK's procedure by 10–20% as against the earlier observation (SZABO, 1970).

In Table 4 the CV and total pigments measured by HPLC in various samples of capsicum extracts are presented. It is interesting to note that the total pigment concentration determined by HPLC and the corresponding MSD–10 CV/1600 value

Table 3

HPLC evaluation of carotenoids distribution in different capsicum extracts

	Sample							
	1	2	3	4	5	6	7	8
Colour Value								
EOA	4 770	14 030	21 838	35 868	54 290	76 128	97 112	104 188
MSD–10	5 042	14 982	23 364	38 280	57 948	81 576	104 280	111 408
ASTA	127	381	561	952	1 490	1 974	2 590	2 738
EOA CV/1600	2.98	8.76	13.64	22.41	33.93	47.58	60.69	65.11
Pigment conc. by BENEDEK's method, g/kg	2.49	7.98	11.81	20.00	29.96	41.50	55.18	58.82
Components (%)								
β-Carotene	3.33	8.56	6.33	12.25	12.60	16.47	9.33	15.24
Non identified	5.04	2.31	1.55	1.06	0.64	0.35	0.22	0.27
Cryptoxanthin	7.23	6.52	8.38	7.84	7.39	8.14	8.04	8.04
like-Lutein	14.51	8.20	12.36	9.63	8.20	8.07	8.57	8.64
trans-Lutein	0.98	1.08	0.77	0.46	0.44	0.51	0.53	0.43
trans-Zeaxanthin	7.90	8.40	8.41	8.05	9.09	9.05	7.05	8.58
Yellows	8.21	5.96	7.28	5.64	5.17	5.48	6.00	5.14
trans-Capsanthin	33.44	38.80	31.69	33.60	35.54	32.08	36.46	33.41
Violaxanthin	4.34	5.39	5.76	5.44	5.22	5.27	5.81	5.16
cis-Capsanthin	12.46	11.97	14.99	13.43	13.08	12.32	14.96	12.52
cis-trans-Capsorubin	2.56	2.81	2.48	2.60	2.63	2.26	3.03	2.57
Red pigments	52.80	58.97	54.92	55.07	56.47	51.93	60.26	53.66

Table 4

CV and HPLC determination of pigments in capsicum extracts

Sample No.	EOA CV	ASTA CV	MSD–10 CV	EOA CV / 1600	ASTA CV / 40	MSD–10 CV / 1600	HPLC Conc. g/kg	(8–5)	(6–8)	(7–8)
1	2	3	4	5	6	7	8	9	10	11
1	118 900	3 188.16	128 000	74.31	79.70	80.00	78.52	4.21	1.18	1.48
2	113 200	3 034.00	121 800	70.75	75.85	76.12	74.85	4.10	1.00	1.27
3	109 400	2 935.60	117 700	68.37	73.39	73.56	72.46	4.09	0.93	1.10
4	95 600	2 568.24	102 900	59.75	64.20	64.31	62.38	2.63	1.82	1.93
5	58 200	1 561.28	62 600	36.37	39.03	39.12	38.07	1.70	0.96	1.05
6	39 600	1 061.08	42 900	24.75	26.52	26.81	25.24	0.49	1.28	1.57

are almost in harmony. The marginal difference between the two values, as listed in column 11 may be attributed to the loss of pigments during saponification (GOVINDARAJAN et al., 1987) and analysis. It may also be noted that the MSD-10 CV/1600 value agrees fairly well with ASTA CV/40 value. Thus, the pigment concentrations calculated by these two conversions approximate the HPLC value.

3. Conclusions

Colour value determined by EOA, ASTA or MSD-10 method hitherto taken for granted as the quality determinant in judging capsicum extracts is now shown to be in error.

The spectrophotometric methods used in the investigation do not guarantee accurate quantification of red and yellow pigments.

The MSD-10 CV divided by 1600 or ASTA CV by 40 provides rapid measurement of total pigment concentration in capsicum extracts.

For quantification of red and yellow pigment groups, one has to resort to HPLC.

*

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FREE RADICAL SCAVENGING ACTIVITY OF METHANOLIC EXTRACT OF SOME CULINARY HERBS

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The scavenging effect of methanolic extracts of some culinary herbs on free radicals and active oxygen species was investigated. Dried culinary herbs – clove, marjoram, cinnamon, oregano and caraway – contain polyphenolic compounds in high concentration (17.7%, 11.2%, 5.8%, 5.2% and 1.3%, respectively). All spice extracts exerted markedly and increased reducing power with increasing dose. Clove showed the strongest reducing power, but lower than that of ascorbic acid at doses of 0.2–1.0 mg cm^{-3}. There was observed a high linear correlation ($r=0.9358$) between the total phenolic content and reducing power of the spice.

Spices showed marked activity as radical scavengers in the experiment using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, indicating that they have effective activities as hydrogen donors and primary antioxidants to react with lipid radicals. The highest DPPH radical scavenging activity was observed in case of clove.

Methanolic extract of culinary herbs also possessed effectiveness in decrease of chemiluminescence intensity arisen in H_2O_2/luminol and adrenalin/luminol system, as well. In both experiments clove was the most effective in scavenging of H_2O_2 and superoxide (O_2^-). These results indicate that methanolic extract of spices are also scavengers of active oxygen species and they are secondary antioxidants. The reducing power and the scavenging effect on free radical and active oxygen species in connection with the total amount of polyphenolic compounds decreased in the following order clove > oregano > cinnamon = marjoram > caraway. The overall antioxidant effect of methanolic extracts of culinary herbs on lipid peroxidation might be attributed to their properties of scavenging free radicals and active oxygen species.

Keywords: culinary herbs, polyphenolic compounds, antioxidant, scavenger, active oxygen species, free radicals, chemiluminescence

Lipid peroxidation is known as one of the major factors in deterioration during storage and processing of food. In addition, it is thought that lipid peroxidation is strongly associated with carcinogenesis, mutagenesis, ageing, and atherosclerosis (FREEMAN & CRAPO, 1982; ISLAM, 1982). The addition of antioxidants has become popular as a means of increasing the shelf life of food products. The recent consumer interest in "natural" products requires natural antioxidative substances to replace conventional antioxidants such as BHT and BHA.

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On the whole, although some antioxidants may be suspected to act as promoters of carcinogenesis (HUANG & FERRARO, 1992), human daily intake of naturally occurring plant phenolic compounds and synthetic antioxidants in food and beverages is considered to have beneficial rather than adverse effects (MACHEIX & FLEURIET, 1994). An antioxidant as food additive should present several essential requirements: effectiveness at low concentration, physical and chemical compatibility with the substrate, absence of sensory influence on the food product and absence of toxic effect (NIKI, 1987).

Antioxidants are generally classified into two groups, namely primary or chain breaking antioxidants which react with lipid radicals to convert them into more stable products, and secondary or preventive antioxidants which reduce the rate of chain initiation or decompose hydroperoxides to non-radical species (LARSON, 1988; YEN & DUH, 1994).

There is a considerable interest in the food industry and in preventive medicine in the development of natural antioxidants from plant materials. Natural antioxidants are primarily plant phenolic compounds which are bioactive substances occurring widely in food plants. Numerous studies have been carried out in order to identify natural phenolics that possess antioxidant activity and some of them have already been extracted from plant sources and are produced commercially (CUVELIER et al., 1994; IGILE et al., 1994; TSUDA et al., 1994).

Almost all of polyphenolic compounds possess several common biological and chemical properties towards the antioxidant activity: (a) the ability to scavenge active oxygen species and other types of free radicals, (b) the ability to scavenge electrophiles, (c) the potential for autoxidation, producing hydrogen peroxide in the presence of certain metals, (d) the ability to chelate metals, (e) the capability to modulate certain cellular enzyme activity and (f) the ability to inhibit nitrosation (HUANG & FERRARO, 1992; YEN & CHEN, 1995).

In particular, phenolic acids (i.e. gallic and caffeic acids and related molecules) and flavonoids (quercetin, rutin, myricetin, luteolin, naringenin, silybin) have shown marked antioxidant activity. The spices and some medicinal plants are well-known for their antioxidant properties, especially rosemary, sage and other species of Labiatae family have been reported to have strong characteristics (KORCZAK et al., 1990; ARMUOA et al., 1992; CUVELIER et al., 1994). Several workers have shown that clove, cinnamon, black pepper exhibited marked antioxidative activities in a variety of food systems (AL-JALAY et al., 1987; RAMANATHAN & DAS, 1993). AL-JALAY and co-workers (1987) also reported that clove in a system of safflower oil in water emulsion exerted potent antioxidative effect. They emphasized that eugenol, a 2-methoxy phenolic derivative in clove, had 90% of the antioxidative activity of butylated hydroxyanisole.

The objective of our work was to investigate the free radical scavenging effect and reducing power of methanolic extract of some spices often used in Hungarian cuisine. Due to the high content of phenolic compounds found in spices, the investigation of the reactivity with free radicals and the measurement of reducing power will be helpful in understanding the mechanism of antioxidant behaviour of these plants.

1. Materials and methods

Dry spices of commercial quality: clove (*Eugenia caryophyllata*), cinnamon (*Cinnamomum cassia*), marjoram (*Majoranna hortensis*), oregano (*Origanum vulgare*) and caraway (*Carum carvi*) were purchased at the local drugstores. 1,1-diphenyl-2-picrylhydrazyl, hemin and catechin from Sigma, luminol reagent from BioOrbit, L-epinephrine (adrenalin) from Calbiochem, butylated hydroxytoluene from Koch-Light Laboratories Ltd. and butylated hydroxyanisole from Fluka were used. All other chemicals were reagents of analytical grade.

Ground, dry spices were defatted two times with 10 volumes of petroleum ether by shaking for 2 h and filtered. The residue was dried under hood until all traces of petroleum ether were removed. Defatted spices (15 g) were shaken for 2 h with 10 volumes of methanol and filtered. The extraction was repeated and the combined filtrates were evaporated in vacuum below 50 °C on a rotary evaporator to a final volume of 15 cm^3. The concentration of the extracts in the solvent of clove, oregano, marjoram, cinnamon and caraway was 208 ± 8.7, 167 ± 8.0, 133 ± 6.7, 112 ± 5.2 and 66 ± 3.9 mg cm^{-3}, respectively.

The concentration of total phenolic compounds of spices was determined using Folin-Denis method (A.O.A.C., 1990). The methanolic extract of spices (0.1 cm^3) was diluted with distilled water to 7 cm^3. Folin-Denis reagent (0.5 cm^3) was added, and the content of the tube was mixed thoroughly. After 3 min, saturated Na_2CO_3 solution (1 cm^3) was added and finally quantified to 10 cm^3 with distilled water. The mixture was allowed to stand for 30 min with intermittent shaking. The blue colour was measured with spectrophotometer at 725 nm. The concentration of total phenolic compounds in the dry spices was determined by comparison with the absorbance of standard catechin at different concentration.

The reducing power of the plant extracts was determined according to the method of OYAIZU (1986). Methanolic extracts of spices (1 cm^3) were mixed with phosphate buffer (2.5 cm^3, 0.2 mol l^{-1}, pH 6.6) and potassium ferricyanide /$K_3Fe(CN)_6$/ (2.5 cm^3, 1%); the mixture was incubated at 50 °C for 20 min. A portion of trichloracetic acid (2.5 cm^3, 10%) was added to the mixture, which was then centrifuged at 3000 rpm, for 10 min. The upper layer of solution (2.5 cm^3) was

mixed with distilled water (2.5 cm^3) and $FeCl_3$ (0.5 cm^3, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated elevated reducing power.

The scavenging effect of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined as described by BLOIS (1958) and modified by HATANO and co-workers (1988). Methanolic extracts of spices (4 cm^3) were added to a methanolic solution of DPPH (1 mM, 1 cm^3). The mixture was shaken and left to stand at room temperature for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm.

For the characterization of the scavenging effect of the plant extracts on hydrogen peroxide and the total scavenging capacity in adrenaline/adrenochrome system the chemiluminescence technique was used (BÖGL & HEIDE, 1984, ZSINKA et al., 1988). The measurements were carried out with a BioOrbit 1251 Luminometer (Turku, Finland). The chemiluminescence intensity was measured and recorded in mV, and the evaluation of a sample takes 2 min. The luminol reagent solution consisted of 0.7 mmol l^{-1} luminol, 38 μmol l^{-1} hemin, 11.8 mmol l^{-1} Na_2CO_3 adjusted to pH 10.7. The total scavenging capacity in adrenaline/adrenochrome system was measured by the evaluation of the decrease of chemiluminescence intensity caused by the reaction between adrenaline (10^{-3} mol l^{-1}, 600 μl) and luminol. The scavenge of hydrogen peroxide was determined by the reaction between hydrogen peroxide (0.88 mmol l^{-1}, 50 μl) and luminol.

Numerical data represent the average and the standard deviation of five measurements. For the statistical evaluation the Student's t-test was used.

2. Results and discussion

The dry spices have high content of total phenolic compounds as it can be seen in Table 1. Clove contains the highest level of the phenolic compounds (17.7%) and lower levels were observed in oregano, marjoram, cinnamon and caraway (11.2%, 5.8%, 5.2% and 1.3%, respectively). Phenolic substances are supposed to be responsible for inhibition of lipid peroxidation (RAMARATHNAM et al., 1988; DECKER & CRUM, 1991). Since the lipid peroxidation is induced and developed by free radicals and active oxygen species, free radical scavengers and oxygen quenchers may inhibit lipid peroxidation. Phenolic compounds that exhibit scavenging efficiency of free radicals are numerous and widely distribution within the plant kingdom (MACHEIX & FLEURIET, 1994).

As shown in Fig. 1, the reducing powers (absorbance at 700 nm) of the plants depended on the concentration of the sample in the reaction mixture. The highest

reducing power was observed in the case of clove related to the high content of phenolic compounds. Reducing powers of the plants are smaller than that of ascorbic acid at 0.2–1.0 mg. The results show that the methanolic extracts of these plants are electron donors and can react with free radicals to convert them to more stable products and terminate radical chain reactions. A high linear correlation coefficient was calculated between phenolic content and reducing power of the plants ($r=0.9358$). The results emphasize the importance of phenolic compounds in the antioxidant behavior of spices.

The scavenging activity of methanolic extract of spices on the 1,1-diphenyl-2--picrylhydrazyl (DPPH) radical decreased in the order clove > oregano > cinnamon > > marjoram > caraway (Table 2). Methanolic extracts of spices mixed with DPPH decolorized DPPH due to their hydrogen donating ability (BLOIS, 1958). Radical scavenging activity of all plants was very high but lower than that of well-known synthetic antioxidants BHA and BHT. The highest DPPH radical scavenging activity was observed in case of clove at a dose of 10.4 μg which scavenged 35.1% of the radicals. Butylated hydroxytoluene showed similar inhibitory effect (38.4%) but at a dose of 2 μg. The scavenging activity on DPPH radicals of the plants depends on their phenolic content, but significant correlation could not be determined.

With respect to results, the methanolic extracts of spices are free radical inhibitors, primary antioxidants that react particularly with the hydroperoxide radicals, which are the major propagator of the chain autoxidation of fats (LEA, 1958), so phenolic compounds can play an important role in breaking the cycle of the lipid peroxidative chain reactions. The carcinogenic property of polyaromatic hydrocarbons (PAH) may be due in part to the in situ generation of PAH cation free radicals (HUANG & FERRARO, 1992). Antioxidants therefore have been reported to possess antimutagenic activity because they could scavenge a free radical or induce antioxidative enzymes (HOCHSTEIN & ATALLAH, 1988).

Table 1

Concentration of total phenolic compounds (%) in dry spices measured by Folin-Denis method

Spices		%
Clove	(*Eugenia caryophyllata*)	17.7
Oregano	(*Origanum vulgare*)	11.2
Marjoram	(*Majoranna hortensis*)	5.8
Cinnamon	(*Cinnamomum cassia*)	5.2
Caraway	(*Carum carvi*)	1.3

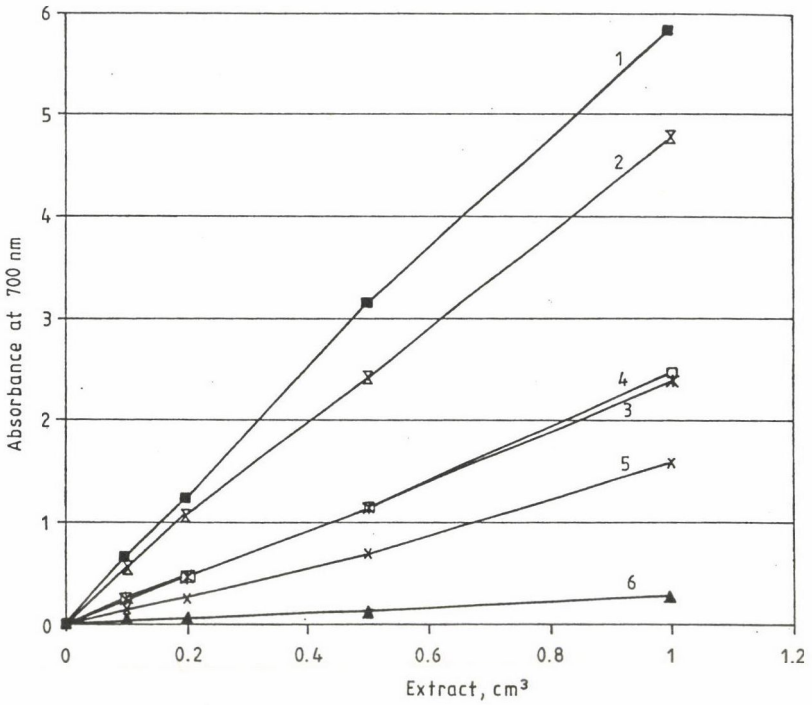

Fig. 1. Reduction power (absorbance at 700 nm) of methanolic extract of spices. Ascorbic acid concentration (1)=1.0 mg cm^{-3}; sample concentration (mg extracted dry material cm^{-3}): clove (2)=2.06, oregano (4)=1.67, cinnamon (3)=1.12, marjoram (5)=1.33, caraway (6)=0.66

Table 2

Scavenging effect of various spice extracts on DPPH radicals

Sample	Concentration ($\mu g\ cm^{-3}$)	Absorbance (517 nm)[a]	Inhibition (%)
Control	–	0.596±0.006 A	–
Clove	10.4	0.387±0.004 B	35.1
Oregano	16.7	0.249±0.019 C	58.2
Cinnamon	11.2	0.306±0.006 D	48.7
Marjoram	13.3	0.361±0.016 B	39.4
Caraway	52.8	0.442±0.005 E	25.8
BHA	2.0	0.367±0.008 B	38.4
BHT	2.0	0.470±0.007 E	21.1
Catechin	3.0	0.249±0.002 C	58.2

[a] Each value is the mean and the standard deviation of five replicate analyses. Values within a column with the same upper case letter are not significantly different at $P<0.05$ probability level

The spice extracts were also capable of scavenging hydrogen peroxide in a concentration dependent manner (Table 3). They exhibited scavenging effect (40–87%) on H_2O_2 in the concentration of 0.41–16.70 $\mu g\ cm^{-3}$. There could not be observed significant correlation between hydrogen peroxide scavenging activity and the phenolic content. Although hydrogen peroxide has only weak activity to initiate lipid peroxidation, but its activity as an active oxygen species comes from its potential to produce the highly reactive hydroxyl radical through the Fenton reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$ (COHEN, 1986). Therefore, the ability of methanolic extract of spices to scavenge hydrogen peroxide may contribute to the inhibition of the lipid peroxidation.

Table 3

Scavenging effect of spice extracts on hydrogen peroxide

Sample	Concentration ($\mu g\ cm^{-3}$)	Chemiluminescence intensity (mV)[a]	Inhibition (%)
Control	-	26.620±1452 A	-
Clove	0.42	7.533± 163 B	71.7
	2.08	3.259± 298 C	87.7
Oregano	1.67	5.416± 68 G	79.6
	16.70	1.744± 788 H	93.4
Marjoram	1.33	5.768± 206 F	78.3
	13.30	4.134± 390 E	84.5
Cinnamon	1.12	15.897± 262 D	40.3
	11.20	4.258± 138 E	84.0
Caraway	0.66	15.817± 259 D	40.5
	6.60	3.203± 131 C	88.0
BHA	2.0	1.314± 21 I	95.1
	10.0	63 ± 8 J	99.8
Catechin	2.0	4.834± 268 K	81.8
	10.0	111± 10 L	99.6

[a] Each value is the mean and the standard deviation of five replicate analyses. Values within a column with the same upper case letters are not significantly different at $P<0.05$ probability level

Table 4

Radical scavenging effect of spice extracts in adrenalin/adrenochrom system

Sample	Concentration ($\mu g\ cm^{-3}$)	Chemiluminescence intensity (mV)[a]	Inhibition (%)
Control	-	5.480±230 A	-
Clove	83.2	4.315± 5 B	21.3
	104.0	3.956±155 C	27.8
Cinnamon	44.8	4.773±212 D	12.9
	56.0	4.358±325 B	20.5
Marjoram	66.5	5.103±130 E	7.0
	266.0	2.908± 36 F	46.9
Oregano	83.5	5.444± 90 A	0.7
	334.0	2.083± 52 G	61.9
Caraway	33.0	5.390±260 A	1.6
	132.0	2.115±331 G	61.4
BHT	2000	4.683±101 D,B	14.5
BHA	2000	1.892± 60 H	65.5
Catechin	2000	344± 41 I	93.7

[a] Each value is the mean and the standard deviation of five replicate analyses. Values within a column with the same upper case letters are not significantly different at $P<0.05$ probability level

The methanolic extract of spices significantly diminished the formation of free radicals in adrenalin/adrenochrom system in the presence of luminol (Table 4). The scavenging effect of the plant extracts was enhanced with increasing concentration. BHT, BHA and catechin also scavenged the radicals in the concentration of 2 mg cm^{-3} (14.5%, 65.5% and 93.7%, respectively) and superoxide anions (O_2^-) were inhibited (46–61%) by spice extracts at concentration of 2 mg cm^{-3}. The scavenging effects of five spice extracts decreased in the order clove > cinnamon > marjoram = oregano = = caraway.

Among other radicals, superoxide originates from adrenaline which directly initiate the lipid peroxidation. Superoxide decomposes to form stronger oxidative species such as singlet oxygen, hydroxyl radical that initiate the lipid peroxidation (McCord & Fridovich, 1978). Superoxide indirectly initiates lipid peroxidation as a result of superoxide and hydrogen peroxide that serve as precursors of singlet oxygen

and hydroxyl radicals. Some researchers reported that superoxide directly initiated lipid peroxidation (FRIDOVICH, 1983). ROBAK and GRYGLEWSKI (1988) indicated that antioxidative properties of several flavonoids such as quercetin, myricetin and rutin were effective mainly via scavenging of superoxide radicals, so the marked antioxidant activity of spices may be concerned with its high activity of scavenging superoxide.

3. Conclusion

Our results demonstrated that spices originally having high content of phenolic compounds bear strong hydrogen-donating activity and they are effective scavengers of free radicals, hydrogen peroxide and superoxide radicals in the order > clove > oregano > cinnamon = marjoram > caraway. These properties seem to be important in explaining how the antioxidant activity of spices originates. The overall antioxidant effect of culinary herbs on lipid peroxidation might be attributed to their properties of scavenging free radicals and active oxygen species.

*

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THE PHYSICO-CHEMICAL PROPERTIES AND COMPOSITION OF HONEYS OF DIFFERENT BOTANICAL ORIGIN

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The concentration of macro- and microelements, the free- and lactone acids, the electrical conductivity and the proline content were measured in honeys of known botanical origin. For the measurement of mineral composition a Thermo-Jarrel-Ash simultaneous multielement ICP–AES spectrometer, type ICAP–9000 was used. The free and lactone acids were determined by an A.O.A.C. titrimetric method. The electrical conductivity was measured in a solution of 20% dry matter content. To analyse the proline content a spectrophotometric method was used.

The results show that floral honeys and honeydew honeys can be distinguished by the measured parameters. Among the floral honeys acacia honey differs the most from the others in its proline and total element content as well as in its free and total acid content.

We used discriminant analysis to distinguish honeys of known botanical origin. For this purpose we considered 12 variables of 28 honey samples of 6 types produced in 1993. We calculated the discriminant functions considering several combinations of the variables. The result was that fructose and glucose content, electrical conductivity and proline content account the most for distinguishing among the groups.

Keywords: honey composition, minerals, proline

According to the Hungarian standard, honeys of different botanical origin are distinguished traditionally by their sensory assessment and pollen content. The sensory assessment of the organoleptic properties is generally subjective and the determination of botanical origin by pollen analysis is confronted with some problems, as

- the relative inaccuracy of the counting,
- the number of "marking" pollen grains in the nectar depends on the anatomy of flowers, the weather, the foraging range, etc. (JONES, 1987; VORWOHL, 1990; GULYÁS, 1991).

The chemical composition of honey is complex and the contents of individual constituents vary considerably (ZANDER & KOCH, 1975; WHITE, 1978). Surveys of floral honey composition have established that the three major components are

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fructose, glucose and water (DONER, 1977). In addition di- and trisaccharides and some higher sugars have also been identified (NIKOLOV et al., 1984; MAUCH et al., 1987; SWALLOW & LOW, 1990; FÖLDHÁZI, 1994).

Honey as a natural product of limited supply and relatively high price, traditionally has been a target for adulteration. The adulteration of honey with various sweet syrups without fear of detection is a great threat to the integrity of the honey market and to the fair treatment of consumers. Several research works were done to develop methods for the detection of honey adulteration (LUCCHESI, 1979; DEIFEL et al., 1985; WHITE et al., 1986; LIPP et al., 1988).

The honey has been suggested as an environmental indicator (BOGDANOV et al., 1986; FODOR & MOLNÁR, 1993). JONES (1987) concluded that the low concentration of heavy metals in honey and their inherent variability detract from the reliable use of honey for monitoring purposes.

In recent years research efforts were done in order to find more characteristic components for classifying honeys (BLANK, 1990; CASTRO et al., 1992; DELGADO et al., 1994).

The aim of our work was to search for significant parameters in distinguishing honeys of different botanical origin.

1. Materials and methods

1.1. Materials

Thirty-eight honey samples (acacia, limetree, sweet chestnut, common milkweed, mixed flower, sunflower, sage, eucalyptus, bears onion, cockshead and honeydew) were collected and measured in 1993, and twenty-one samples (acacia, limetree, common milkweed, sweet chestnut, sunflower, phacelia) in 1994.

1.2. Methods

Moisture determination was performed by measuring the refractive index value (Abbé refractometer at 20 °C). Average refractive index values were converted to honey moisture contents using the table developed by Wedmore (A.O.A.C., 1990a).

For the measurement of mineral composition a Thermo-Jarrel-Ash simultaneous multi-element ICP–AES spectrometer, type ICAP–9000 was used. The typical instrument conditions were: 1.05 kW forward power, 14 l min^{-1} plasma argon, 0.4 l min^{-1} nebuliser argon, Babington type nebuliser (high solid). The instrument is capable of selecting among 26 channels. The integration time was 7 s, using a background correction on both sides. Inter-element correction was used in the

measurement program. Three parallel measurements were made for each sample (FODOR & MOLNÁR, 1993).

Sample preparation for determination of mineral composition was carried out according to A.O.A.C. (1990c). The ash of the 5–10 g honey was diluted with 10 cm^3 0.1 mol HNO_3.

The electrical conductivity was measured in a 20% solution (regarding the dry substance of the honey) at 20 °C using a conductometer (VORWOHL, 1964) and was expressed as S cm^{-1}.

We used a titrimetric method to analyse the acidity (free, lactone and total acid) of the samples (A.O.A.C., 1990b).

The proline content was measured by the German standard method (DIN, 1991). The principle of the method is that proline reacts with ninhydrin solution to form colored compounds. The concentration is determined with a spectrophotometer using a wavelength of 520 nm and a water blank.

Discriminant analysis was carried out by an SPSS/PC + 4.0 software package.

2. Results and discussion

The results of the moisture and pollen content and the sugar composition of the samples harvested during 1993 had been previously published (FÖLDHÁZI, 1994). The results related to the subsequent new parameters are summarised in Tables 1–9.

Table 1 shows both the Mineral composition of honey samples harvested during 1993 and the Recommended Dietary Allowances. Among macro elements potassium content was the highest in all honey samples.

Comparing the honeys of different botanical origin it can be concluded that acacia honey contains the least macro elements while honeydew honey has the most. Considering the macro elements, the limetree and sweet chestnut honeys have been ranked between acacia and honeydew honeys. The average potassium content of sweet chestnut honeys is nearly the same as in limetree honeys, while their Ca, Mg, Na, and P contents are higher. These values are similar to the findings of ZANDER and KOCH (1975), WHITE (1978), MORSE and LISK (1980) and GULYÁS and MOLNÁR (1989).

In the samples investigated the following micro elements were found: Fe, Cu, Zn and Mn. Although the essential Cu and Zn above the Maximal Residue Tolerance Limits (MRL) are considered toxic and hazardous (BOGDANOV et al., 1986; JONES, 1987; SOHÁR, 1992) concentrations below this level has been found in our measurements. In RIGÓ's (1992) opinion in Hungary the diet contains only the 62% of Fe, 55% of Zn, 32% of Cu, and 28% of Mn of the Recommended Dietary Allowance.

JONES (1987) analysing 76 English honey samples found similar quantity of Cu, Pb and Cd as we had. BOGDANOV and co-workers (1986) determined the Zn, Pb and Cd content of 18 Swiss nectar and 21 honeydew honeys. Their findings were similar to ours, too. MORSE and LISK (1980) found 22–183 mg/kg Fe in samples of El Salvador and China and 22–172 mg/kg Zn in Chinese samples. These values are 50–200 times more for Fe and 7–50 times more for Zn than our ones. It is supposed that the high Fe and Zn concentrations come from the contact with metal containers. The Pb content of the samples was 6–15 times higher than in ours. BONVEHI and COLL (1993) analysing 26 Spanish lavender honey samples found similar Mn and Zn concentration and 2 times higher Cu and 5 times higher Fe content. The average Pb content of honeys produced in Connecticut (HILL et al., 1981) was 0.11 mg/kg versus 0.25 mg/kg in our samples. VARJÚ (1970) measured the Pb content of 12 Hungarian acacia honeys produced in 1966 and found 0.05 mg/kg, which is almost one fifth of our findings in 1993.

Tables 2–5 show the Mineral composition of the individual honey samples. These tables contain the macro and micro element contents, compiled by the average and standard deviation data.

Table 2 shows the Mineral composition of the individual acacia honeys. VARJÚ (1970) found 5 times more Ca, 3 times more Mg and 2.5 times more Fe as we did, but the average of our findings are in the range of his results. Our results are in agreement with the findings of WHITE (1978) for light honeys.

Table 3 shows the Mineral composition of the individual limetree honeys. Comparing the limetree honeys with the acacia honeys they contain 2 times more K, 3 times more Ca and 1.5 times more P. Their Fe and Cu contents are less, the average Pb content is double than those of acacia honeys. The Pb content in sample L6 is almost as much, and in L4 is higher than it is allowed for white sugar (FAO/WHO; 1984).

Table 4 shows the Mineral composition of the individual sweet chestnut honeys. Their Fe, Zn, Si, Al, and Mn contents and all macro elements except K were higher than those of acacia and limetree honeys.

Table 5 shows the Mineral composition of the individual honeydew honeys. Comparing honeydew honeys with nectar honeys the honeydew honey's K, P and Mg contents are higher. Except for Fe and P contents the results are similar to WHITE's (1978) findings for dark honeys.

Table 1

Mineral composition of honeys of 1993 (mg/kg)

	Acacia (n=7)		Limetree (n=6)		S.chestnut (n=5)		Honeydew (n=3)		RDA
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	(mg/day)
Macroelements									
K	166.9	42.6	326.2	116.9	319.2	72.9	1319.7	567.0	1875–5626
Na	41.3	8.1	44.1	7.6	59.3	8.2	51.9	6.8	1100–3300
Ca	33.3	10.3	100.1	14.5	143.5	26.0	42.9	13.6	800.00
Mg	4.9	2.1	13.1	4.2	23.2	5.8	32.1	10.9	350.00
P	30.0	7.5	52.5	4.7	89.0	24.0	149.1	59.2	
Microelements									
Fe	1.07	1.86	0.41	0.34	1.37	0.68	0.59	0.23	10 m 18 fm
Cu	0.64	0.46	0.36	0.76	0.62	0.15	0.42	0.15	2–3
Zn	3.25	1.69	3.10	1.92	3.65	2.29	2.18	0.43	15.00
Mn	0.14	0.10	0.52	0.27	1.78	0.79	1.75	0.69	2.5–5
Cr	0.16	0.23	n.d.	–	0.28	0.15	0.29	0.18	0.05–0.20
B	2.42	0.88	3.44	1.98	3.52	0.41	2.72	0.44	
Li	n.d.	–	n.d.	–	0.14	0.09	0.02	0.00	
Si	3.15	2.62	6.88	3.09	9.61	1.48	22.64	13.09	
Al	0.65	0.30	0.69	0.09	1.79	1.02	3.26	2.73	
Ba	0.31	0.22	0.37	0.16	0.40	0.12	0.23	0.10	
Sr	0.19	0.18	0.21	0.04	0.29	0.11	0.14	0.07	
Pb	0.24	0.25	0.49	0.50	0.17	0.15	0.16	0.08	3 PTWI
Cd	n.d.	–	n.d.	–	n.d.	–	n.d.	–	0.5 PTWI

n: number of samples
$\bar{x}$: mean value of fresh sample
s: standard deviation
RDA: recommended dietary allowances
PTWI: provisional tolerable weekly intake

Table 2

Mineral composition of acacia honeys (mg/kg)

	A1	A2	A3	A4	A5	A6	A7	$\bar{x}$	s	min	max
Macroelements											
Ca	36.9	38.9	47.6	20.4	19.4	32.0	38.2	33.3	10.3	19.4	47.6
K	105.4	157.4	155.8	134.3	173.9	222.3	219.0	166.9	42.6	105.4	222.3
Mg	6.2	5.5	6.1	2.2	1.7	7.0	5.8	4.9	2.1	1.7	7.0
Na	42.9	54.9	40.1	31.7	33.0	39.1	47.4	41.3	8.1	31.7	54.9
P	35.2	27.3	35.0	18.1	22.1	36.3	35.8	30.0	7.5	18.1	36.3
Microelements											
Fe	0.50	0.39	0.50	5.31	0.15	0.32	0.37	1.08	1.87	0.15	5.31
Cu	1.39	0.62	0.65	0.23	0.10	0.41	1.09	0.64	0.46	0.10	1.39
Zn	4.64	1.76	4.97	3.88	1.01	4.75	1.72	3.25	1.69	1.01	4.97
Mn	0.14	0.03	0.30	0.08	0.06	0.11	0.23	0.14	0.10	0.03	0.30
Co	0.37	0.10	0.03	0.00	0.00	0.00	0.04	0.08	0.13	0.00	0.37
Cr	0.39	0.56	0.14	0.01	0.00	0.00	0.01	0.16	0.23	0.00	0.56
B	1.68	2.03	2.24	1.92	2.03	4.28	2.74	2.42	0.88	1.68	4.28
Si	1.87	2.14	3.20	1.04	1.30	8.64	3.87	3.15	2.62	1.04	8.64
Al	1.08	0.97	0.57	0.24	0.37	0.59	0.70	0.65	0.30	0.24	1.08
Cd	0.02	0.00	0.02	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.02
Pb	0.71	0.05	0.29	0.09	0.09	0.07	0.40	0.24	0.25	0.05	0.71
Sr	0.20	0.23	0.59	0.06	0.07	0.10	0.10	0.19	0.18	0.06	0.59
Ba	0.28	0.34	0.65	0.06	0.54	0.10	0.19	0.31	0.22	0.06	0.65

A1–A7: sample number
$\bar{x}$: mean value
s: standard deviation

Table 3

Mineral composition of limetree honeys (mg/kg)

	L1	L2	L3	L4	L5	L6	$\bar{x}$	s	min	max
Macroelements										
Ca	100.2	104.4	79.8	86.9	118.6	110.4	100.1	14.5	79.8	118.6
K	392.6	299.3	152.3	238.1	423.6	451.4	326.2	116.9	152.3	451.4
Mg	19.8	11.6	10.4	8.3	16.2	12.2	13.1	4.2	8.3	19.8
Na	43.2	36.9	36.9	42.4	48.8	56.6	44.1	7.6	36.9	56.6
P	51.3	53.2	56.4	43.8	56.5	53.9	52.5	4.7	43.8	56.5
Microelements										
Fe	0.57	0.23	0.37	0.00	0.28	0.99	0.41	0.34	0.00	0.99
Cu	0.00	0.00	0.00	1.91	0.13	0.13	0.36	0.76	0.00	1.91
Zn	2.81	2.19	1.19	6.35	4.34	1.75	3.10	1.92	1.19	6.35
Mn	0.56	0.52	0.98	0.17	0.56	0.35	0.52	0.27	0.17	0.98
Co	0.11	0.08	0.02	0.03	0.03	0.04	0.05	0.03	0.02	0.11
B	3.97	2.60	1.98	3.37	5.00	3.75	3.44	1.06	1.98	5.00
Li	0.19	0.05	0.10	0.08	0.05	0.01	0.08	0.06	0.01	0.19
Si	8.47	4.59	3.22	4.85	11.11	9.06	6.88	3.09	3.22	11.11
Al	0.84	0.60	0.64	0.69	0.75	0.61	0.69	0.09	0.60	0.84
Pb	0.15	0.22	0.11	1.26	0.21	0.98	0.49	0.50	0.11	1.26
Sr	0.21	0.20	0.21	0.18	0.28	0.21	0.21	0.04	0.18	0.28
Ba	0.33	0.32	0.26	0.69	0.39	0.25	0.37	0.16	0.25	0.69

L1–L6: sample number
$\bar{x}$: mean value
s: standard deviation

Table 4

Mineral composition of sweet chestnut honeys (mg/kg)

	C1	C2	C3	C4	C5	$\bar{x}$	s	min	max
Macroelements									
Ca	177.5	126.9	146.8	155.9	110.2	143.5	26.0	110.2	177.5
K	274.4	233.7	313.5	350.6	423.6	319.2	72.9	233.7	423.6
Mg	28.8	21.8	16.5	19.4	29.6	23.2	5.8	16.5	29.6
Na	68.0	56.9	46.5	62.7	62.5	59.3	8.2	46.5	68.0
P	119.9	62.9	68.1	89.6	104.2	89.0	24.0	62.9	119.9
Microelements									
Fe	2.54	1.18	1.28	1.07	0.77	1.37	0.68	0.77	2.54
Cu	1.38	0.33	0.32	0.46	0.60	0.62	0.44	0.32	1.38
Zn	7.65	1.85	3.22	2.88	2.66	3.65	2.29	1.85	7.65
Mn	0.50	2.35	1.96	2.48	1.59	1.78	0.79	0.50	2.48
Co	0.14	0.02	0.06	0.20	0.01	0.09	0.08	0.01	0.20
Cr	0.12	0.33	0.25	0.51	0.18	0.28	0.15	0.12	0.51
B	3.90	2.85	3.53	3.53	3.80	3.52	0.41	2.85	3.90
Li	0.03	0.07	0.15	9.22	0.23	0.14	0.09	0.03	0.23
Si	11.90	9.43	7.75	9.47	9.49	9.61	1.48	7.75	11.90
Al	1.33	1.14	1.33	1.56	3.60	1.79	1.02	1.14	3.60
Pb	0.43	0.10	0.13	0.07	0.11	0.17	0.15	0.07	0.43
Sr	0.42	0.21	0.17	0.37	0.27	0.29	0.11	0.17	0.42
Ba	0.28	0.32	0.37	0.53	0.53	0.40	0.12	0.28	0.53

C1–C5: sample number
$\bar{x}$: mean value
s: standard deviation

Table 5

Mineral composition of honeydew honeys (mg/kg)

	H1	H2	H3	$\bar{x}$	s	min	max
Macroelements							
Ca	30.6	57.5	40.7	42.9	13.6	30.6	57.5
K	1927.5	1226.8	805.0	1319.7	567.0	805.0	1927.5
Mg	20.2	41.4	34.8	32.1	10.9	20.2	41.4
Na	58.5	52.4	44.9	51.9	6.8	44.9	58.5
P	205.0	155.3	87.0	149.1	59.2	87.0	205.0
Microelements							
Fe	0.57	0.82	0.37	0.59	0.23	0.37	0.82
Cu	0.60	0.34	0.33	0.42	0.15	0.33	0.60
Zn	1.78	2.14	2.64	2.18	0.43	1.78	2.64
Mn	1.07	2.44	1.74	1.75	0.69	1.07	2.44
Cr	0.46	0.11	0.29	0.29	0.18	0.11	0.46
B	3.06	2.88	2.22	2.72	0.44	2.22	3.06
Si	35.19	23.67	9.07	22.64	13.09	9.07	35.19
Li	0.12	0.07	0.05	0.08	0.03	0.05	0.12
Al	6.25	2.65	0.89	3.26	2.73	0.89	6.25
Pb	0.17	0.22	0.11	0.16	0.05	0.11	0.22
Ba	0.13	0.33	0.22	0.23	0.10	0.13	0.33
Sr	0.08	0.22	0.13	0.14	0.07	0.08	0.22

H1–H3: sample number
$\bar{x}$: mean value
s: standard deviation

Table 6 shows the mineral content of honeys produced in 1994. The predominant mineral element is also potassium. The composition of macro elements is very similar in acacia, common milkweed, sunflower and phacelia honeys. Higher amounts of potassium were found in limetree and mixed floral honeys. The highest amounts could be measured in sweet chestnut and in pine honeys. Thirteen–fourteen different micro elements could be detected, too. Some of them are essential (Fe, Cu, Zn, Mn), some others could be hazardous (Pb, Cd) in high concentrations. Based on an average honey consumption in Hungary (2–3 g/person/day) and compared with the Recommended Dietary Allowances the intake of essential elements from honey is negligible (MERTZ, 1987).

To consume as much lead as Provisional Tolerable Weekly Intake allows, one could eat 5–30 kg honey/week. The cadmium concentration was at the limit of the analytical sensitivity. It means that no health hazards are expected for honey consumers.

Comparison was made with the data resulting from 1993 and 1994. The average mineral concentrations among honeys of the same botanical origin have not significantly differed from each other. The concentrations of metals found in the investigated samples were comparable to those values reported in the literature.

Table 7 shows the electrical conductivity of honeys produced in 1993. The electrical conductivity first of all depends on the mineral content of the honey. Nectar honeys have lower mineral content than honeydew honeys, so measuring their electrical conductivity they can be distinguished. VORWOHL (1964) suggests using the method for detecting adulteration with sugar feeding honey. Considering the data, it can be seen that the electrical conductivity of common milkweed, acacia and sunflower honeys are between $1.77–2.42 \times 10^{-4}$ S cm^{-1} and that of limetree honeys 8.40×10^{-4} S cm^{-1}. KRAUZE and ZALEWSKI (1991) found 1.55×10^{-4} S cm^{-1} for acacia honey and 6.44×10^{-4} S cm^{-1} for limetree honey. Sweet chestnut and honeydew honeys showed high (11.85×10^{-4} S cm^{-1} and 14.96×10^{-4} S cm^{-1}) electrical conductivity. VORWOHL (1964) measured $8.30–13.63 \times 10^{-4}$ S cm^{-1} for sweet chestnut honeys and $10.36–16.24 \times 10^{-4}$ S cm^{-1} for honeydew honeys. According to KISS (1994) limetree and sweet chestnut honeys always contain a part of honeydew honey.

Table 8 shows the proline content of honeys produced in 1993. Among free amino acids proline predominates representing 50–85% of the total (WHITE, 1978). DAVIES (1976), using data for 98 honey samples has suggested that certain ratios between contents of various amino acids could be used for determining the geographic source of a honey. He found that while there are variations in the ratios between samples in the same area, the variation between sources is much greater. LUCCHESI (1979) proposed using proline content as an indicator of honey purity, but the wide range of values found precluded the usability of it.

Table 6

Mineral composition of honeys of 1994 (mg/kg)

	Acacia	(n=8)	C.milkweed	(n=3)	Limetree	(n=2)	S.chestnut	(n=3)	Sunflower	(n=3)	Phacelia	(n=2)	Rape	Pine	Flower
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	x(n=1)	x(n=1)	x(n=1)
Macroelements															
K	140.6	43.9	169.4	117.0	280.2	148.1	698.3	359.1	126.3	8.5	105.4	15.0	86.6	740.1	291.3
Na	24.2	7.5	24.6	16.2	32.3	8.5	22.3	13.4	24.6	8.0	23.7	5.0	14.1	32.4	40.5
Ca	32.3	6.1	31.1	23.9	70.5	41.7	125.0	27.5	82.4	4.6	25.1	6.4	59.9	60.2	122.5
Mg	6.9	1.6	11.9	9.5	17.6	5.0	22.4	10.5	19.3	4.8	4.7	1.2	17.7	79.8	32.1
P	35.0	7.9	34.9	25.0	49.4	13.1	63.4	10.0	53.7	11.0	31.8	9.5	43.3	162.4	67.5
Microelements															
Fe	0.78	0.35	2.03	0.27	1.41	1.03	1.79	2.03	1.02	0.33	0.71	0.22	0.92	2.15	6.72
Cu	0.48	0.39	0.45	0.20	0.27	0.10	0.50	0.50	0.35	0.18	0.45	0.39	0.39	1.54	0.37
Zn	2.07	1.72	2.77	1.98	5.80	4.65	2.83	2.93	3.35	0.65	1.44	0.27	3.91	2.75	13.09
Mn	0.12	0.06	0.28	0.20	0.63	0.33	4.46	3.43	0.21	0.10	0.07	0.00	0.15	3.65	0.87
Cr	0.20	0.09	0.27	0.06	0.11	0.06	0.56	0.32	0.26	0.13	0.29	0.01	0.35	0.18	0.14
B	1.12	0.49	1.38	0.10	2.25	0.46	2.32	0.92	2.82	1.51	0.98	0.68	2.35	2.17	2.74
Li	0.07	0.04	0.21	0.20	0.05	0.04	0.12	0.04	0.07	0.01	0.08	0.05	0.05	0.07	0.13
Si	7.63	3.98	11.49	0.66	8.74	3.28	15.71	4.16	10.79	4.06	4.29	0.90	6.25	23.27	11.82
Al	0.60	0.13	0.72	0.32	0.84	0.17	1.09	0.45	0.83	0.31	0.46	0.10	0.69	10.1	1.40
Ba	0.17	0.08	0.33	0.20	0.16	0.04	0.53	0.21	0.16	0.04	0.20	0.08	0.38	0.34	0.25
Co	0.10	0.12	0.43	0.24	0.16	0.03	0.26	0.34	0.06	0.01	0.02	0.01	0.03	0.27	0.75
Ni	0.11	0.09	0.18	0.10	0.04	0.01	0.16	0.21	0.05	0.02	0.05	0.03	0.03	0.54	0.16
Sr	0.09	0.03	0.15	0.09	0.12	0.02	0.22	0.06	0.08	0.03	0.09	0.01	0.1	0.16	0.15
Pb	0.13	0.04	0.30	0.19	0.11	0.03	0.18	0.08	0.16	0.02	0.13	0.01	0.1	0.39	0.21

n: number of samples

$\bar{x}$: mean value of the fresh sample

s: standard deviation

Table 7

The electrical conductivity of honeys (10^{-4}S cm^{-1})

	$\bar{x}$	min	max
Common milkweed (n=3)	1.77	1.72	1.80
Acacia (n=9)	1.86	1.62	2.17
Sunflower (n=3)	2.42	2.05	2.80
Limetree (n=7)	8.40	6.10	9.50
Sweet chestnut (n=4)	11.85	8.50	15.05
Honeydew (n=3)	14.96	13.20	18.00

n: number of samples
$\bar{x}$: mean values
min, max: range of measured values

Our results show that among the analysed samples the proline content of acacia honeys are the lowest (199 mg/kg), which is similar to the value mentioned in the German standard (171 mg/kg), and agrees with the findings of KRAUZE and ZALEWSKI (1991). The proline content of common milkweed and sunflower honeys are 50% higher. The proline content of limetree honey seems to be double than that of the acacia one. KRAUZE and ZALEWSKI (1991) measured similar values in limetree and honeydew honeys, too (424 mg/kg and 606 mg/kg, respectively).

Table 9 shows the free acid and the gluconolactone concentration of honeys produced in 1993. Gluconic acid in equilibrum with gluconolactone is the principal acid of honey. It is produced from glucose by glucose oxidase (WHITE, 1978). Among the analysed samples acacia honey's acid concentration is the lowest and honeydew honey's is the highest. Our findings are in the range with the data of WHITE (1978) and BONVEHI and COLL (1993).

Table 8

Proline content of honeys (mg/kg)

	$\bar{x}$	min	max
Acacia (n=9)	199	189	283
Common milkweed (n=3)	305	242	345
Sunflower (n=2)	419	374	464
Limetree (n=7)	426	337	495
Honeydew (n=4)	563	487	608
Sweet chestnut (n=4)	733	662	943

n: number of samples
$\bar{x}$: mean value
min, max: range of measured values

Table 9

Free acid, lactone and total acid of honeys (mEq/kg)

	Free acid		Lactone		Total acid	
	$\bar{x}$	min–max	$\bar{x}$	min–max	$\bar{x}$	min–max
Acacia (n=9)	4.98	3.87– 5.91	3.39	1.53– 4.58	8.38	5.4–10.02
Common milkweed (n=3)	9.40	7.0 –11.8	4.90	3.0 – 8.7	14.30	10.1–20.5
Sweet chestnut (n=4)	10.33	11.3 – 9.3	4.20	6.6 – 1.8	14.50	18.0–11.1
Limetree (n=7)	11.71	6.34–16.7	6.64	2.0 –10.3	18.35	8.4–24.8
Sunflower (n=2)	14.20		10.90		25.10	
Honeydew (n=3)	25.70	22.5 –28.9	5.60	5.11– 6.1	31.30	28.6–34.1

n: number of samples

$\bar{x}$: mean values

min, max: range of measured values

Authors made efforts to make classification among honeys using discriminant analysis (DA). The essential purpose of DA is classification of an item into one of the several mutually exclusive groups on the basis of its measured response variables (PIGOTT, 1986; NORUSIS, 1990). It is thus of special value to predict the class to which unknown samples belong.

In our work we used several combination of the variables to find the ones which would be the most strongly associated with the honey types. When we considered all the 12 variables, total acid content failed the tolerance test. Total acid content is in a very good correlation with the free acid content. Among the discriminant functions calculated from the remaining 11 variables, the first accounts for 85.62% and the second for 7.73% of the total between-groups variability. Figure 1 presents the separation of the first two discriminant functions.

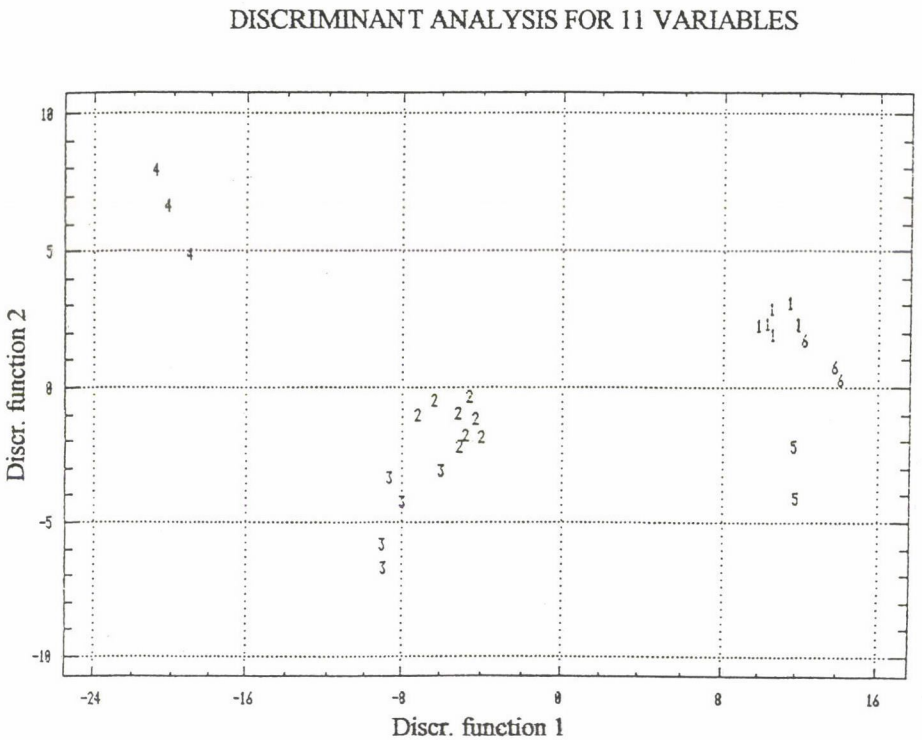

Fig. 1. Scatterplot of the values of the first two discriminant scores for 28 honey samples. The variables: fructose, glucose, erlose, melecitose, potassium, total macro elements, pH, free acid, lactone, electrical conductivity and proline. Discriminant function 1=85.62%, discriminant function 2=7.73%. 1: acacia honey; 2: limetree honey; 3: sweet chestnut honey; 4: honeydew honey; 5: sunflower honey; 6: common milkweed honey

Among the well separated groups acacia and common milkweed honeys as well as limetree and sweet chestnut honeys are close to each other. The first two functions have a good relationship with the honey groups, none of the samples was misclassified.

We investigated the possibility to reduce the number of measurements. In the first case the variables fructose, glucose, erlose and melecitose contents are measured by HPLC and free acid and lactone by titration (only two measurements). Among the discriminant functions calculated from the 6 variables the first accounts for 63.46% and the second for 19.58% of the between-groups variability. The third discriminant function accounts for 14.71%. In this calculation 26 out of 28 cases (92.86%) are correctly classified. The results are presented in Fig. 2 and Fig. 3.

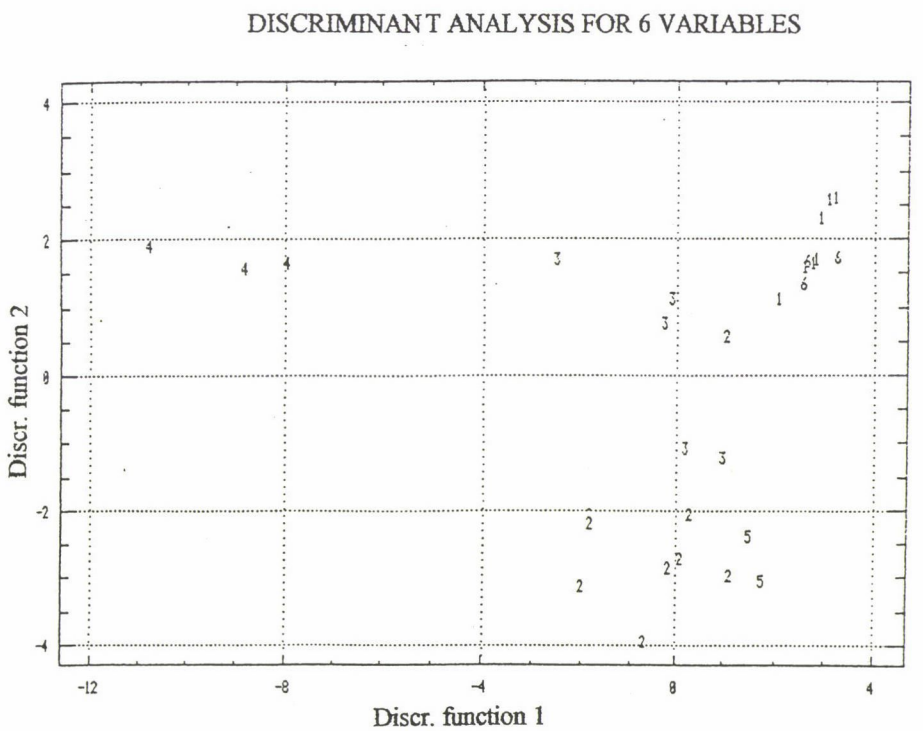

Fig. 2. Scatterplot of the values of the first two discriminant scores for 28 honey samples. The variables: fructose, glucose, erlose, melecitose, free acid and lactone. Discriminant function 1=63.46%; discriminant function 2=19.58%. 1: acacia honey; 2: limetree honey; 3: sweet chestnut honey; 4: honeydew honey; 5: sunflower honey; 6: common milkweed honey

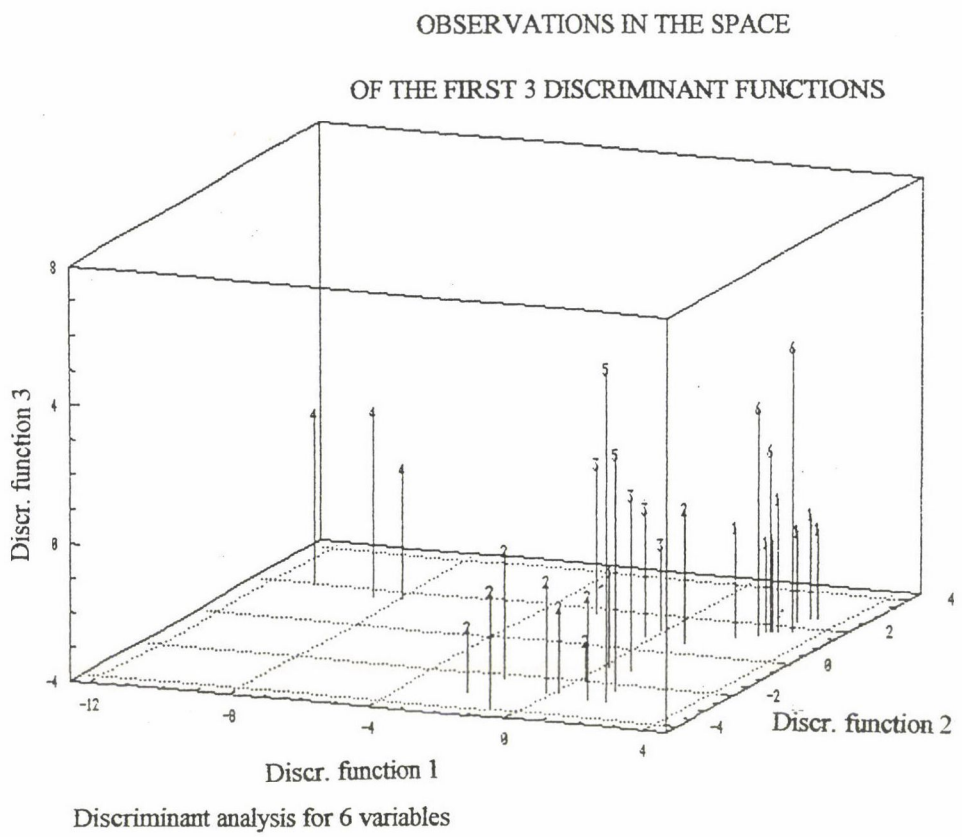

Fig. 3. Scatterplot of the values of the first three discriminant scores for 28 honey samples. The variables: fructose, glucose, erlose, melecitose, free acid and lactone. Discriminant function 1=63.46%; discriminant function 2=19.58%, discriminant function 3=14.71%. 1: acacia honey; 2: limetree honey; 3: sweet chestnut honey; 4: honeydew honey; 5: sunflower honey; 6: common milkweed honey

We show the results of the first 3 discriminant functions in Fig. 3.

Considering 4 variables: fructose and glucose content, electrical conductivity and proline content (it needs three measurements), the first discriminant functions are 71.23%, 22.82% and 5.87% respectively. Figure 4 shows the results in the level of the first two functions, while Fig. 5 presents the results in the first three discriminant functions.

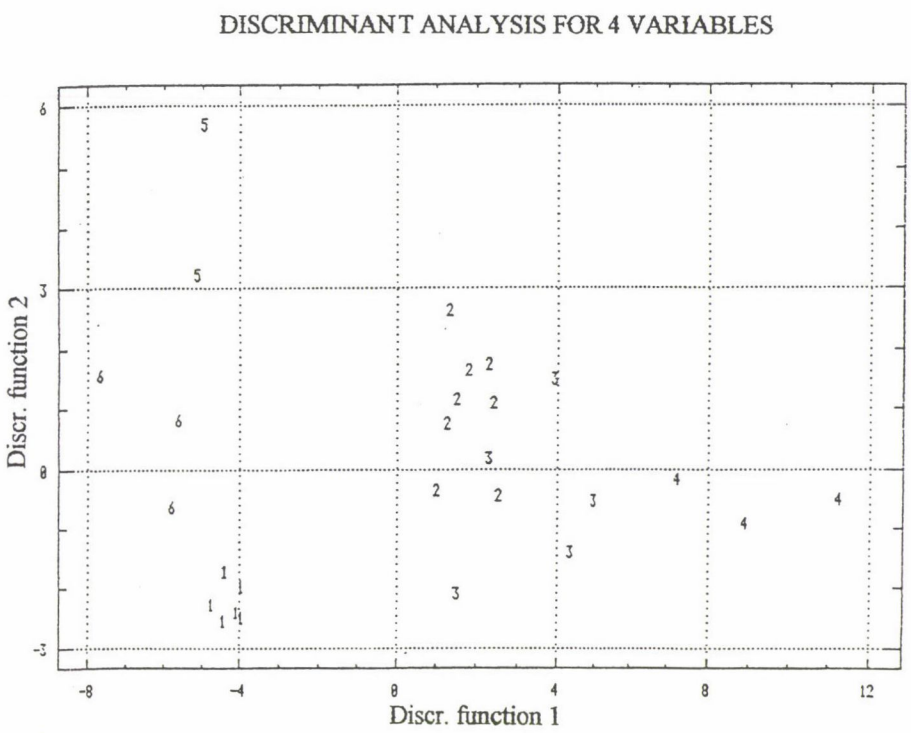

Fig. 4. Scatterplot of the values of the first two discriminant scores for 28 honey samples. The variables: fructose, glucose, electrical conductivity and proline. Discriminant function 1=71.23%, discriminant function 2=22.8%. 1: acacia honey; 2: limetree honey; 3: sweet chestnut honey; 4: honeydew honey; 5: sunflower honey; 6: common milkweed honey

The separation of the different honeys is nearly as good as showed in Fig. 1 where 11 variables were considered. None of the samples was misclassified.

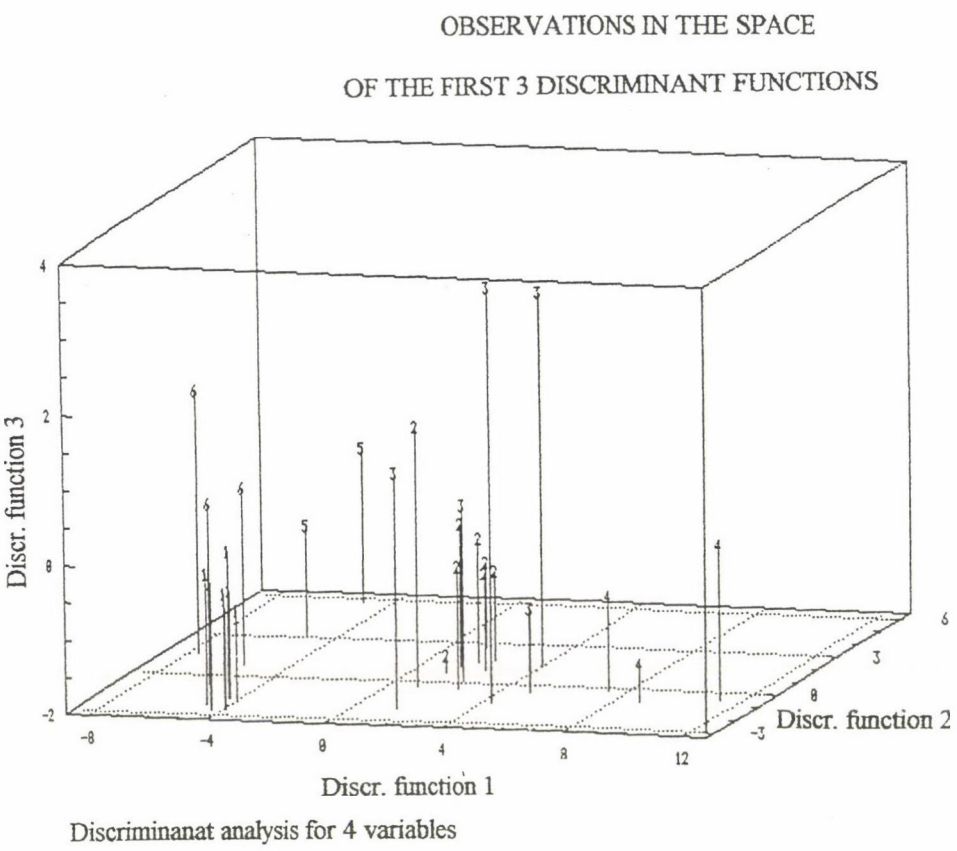

Fig. 5. Scatterplot of the values of the first three discriminant scores for 28 honey samples. The variables: fructose, glucose, electrical conductivity and proline. Discriminant function 1=71.23%, discriminant function 2=22.82%, discriminant function 3=5.87%. 1: acacia honey; 2: limetree honey; 3: sweet chestnut honey; 4: honeydew honey; 5: sunflower honey; 6: common milkweed honey

3. Conclusion

The concentrations of metals found in the honey samples collected in Hungary were found to be comparable to values reported internationally. The content of heavy metals are below hazardous concentrations (MRD). The related values of individual samples of both nectar and honeydew honeys covered wide ranges, but the average amounts among honeys of the same botanical origin produced in 1993 and 1994 are similar.

Honeys of different botanical origin differ from each other in chemical composition. Among the analysed samples acacia honey contains the least amount of minerals, proline and total acids. Nectar honeys and honeydew honeys can be distinguished by the monosaccharide and trisaccharide composition and quantity (FÖLDHÁZI, 1994) as well as by their electrical conductivity and the contents of macro elements.

The proline content in experimental acacia honey, limetree honey, sweet chestnut honey and honeydew honey are consistent with data cited in the literature.

The DA shows that analysing the fructose and glucose content, electrical conductivity and the proline contents and calculating the discriminant functions, the first two account for 94.05% of the between-groups variability. To get more reliable results more honey samples of known botanical origin should be analysed and calculated via DA before using it to predict the type of unknown samples.

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CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF THE CULTIVATED (*AGARICUS BISPORUS*) AND WILD MUSHROOMS GROWN IN TURKEY

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The chemical composition (moisture, ash, protein, fat, total sugars, water soluble vitamins – vitamin B_1, B_2, C, folic acid, pantothenic acid and vitamin PP, and minerals – Zn, Cu, K, Na, Fe, Ca, Cr and P) of cultivated mushrooms (*Agaricus bisporus*) obtained from three different commercial firms and wild mushrooms grown in Turkey have been determined.

Some chemical constituents of the cultivated mushrooms are as follows (as mean values of three different samples; %): moisture 92.43; protein 2.39; total sugars 0.23; fat 0.56; ash 0.76. Mineral contents (ppm) of samples were found to be: Zn 41.23; Cu 7.10; K 1513.61; Na 49.94; Fe 7.90; Ca 30.45; Cr trace; P 923.2. Vitamin contents of samples were (mg/100 g): B_1 0.077; B_2 0.417; folic acid 0.058; C 5.60; pantothenic acid 2.07; PP 5.32.

The results for some edible wild mushrooms (*Morchella esculanta* and *Lactarius deliciosus*) showed that variation in chemical constituents were mainly dependent on the type of mushroom.

Keywords: mushroom, nutrients

Mushroom, a food obtained with little human involvement, is a natural food containing several nourishing principles. The mushrooms are divided into edible and poisonous kinds. Although only twenty-five species out of over 2000 edible mushrooms are widely accepted as food, and only few of them attract big attention as items of commerce, and despite the fact that people pay a very high price for them, there is a demand for the less common edible wild mushrooms in European Community countries where the people like exotic foods containing wild mushrooms (ZAKHARY et al., 1983).

The cultivation and consumption of edible mushrooms of which *Agaricus bisporus* is the most important (Table 1), have increased in recent years along with an increased emphases on improving the quality and availability of fresh produce in the world (HADAR & DOSORETZ, 1991). There are over one million tons of mushrooms produced worldwide, the bulk of which are *Agaricus bisporus* – the button mushroom

0139-3006/96/$ 5.00

– (65%), followed by *Lentinus edodes* – the Shiitake mushroom – (16%) and *Volvariella volvacea* – the straw mushroom – (6%).

Between the early 1970s and the late 1980s, in a span of about 18 years, the production of cultivated mushrooms in Turkey doubled. This increase indicates a growing interest in cultivated mushrooms, of which *Agaricus bisporus* is the main species. It has become easily obtainable and is popularly eaten raw and cooked in a variety of ways. Also it is partly exported to EC countries.

DUDA and co-workers (1980), KIKUCHI and co-workers (1984), KOSSON and BAKOWSKI (1984), KURASAWA and co-workers (1982), LEVAI (1989), MAU and co-workers (1991), MITCHELL and SAVAGE (1990), STANTON (1984), TSAI and co-workers (1974) and ZAKHARY and co-workers (1983) reported that *Agaricus bisporus* supplies a slightly higher percentage of protein, containing all the essential amino acids, compared with vegetables. It is low in calories and is a good source of water soluble vitamins, especially B complex vitamins, thiamin, riboflavin, niacin, pantothenic acid, and minerals, especially iron, phosphorus and potassium. On the other hand, according to data in the literature the chemical composition of *Agaricus bisporus* is effected by cultivation methods, the type of spawn and the composition of the compost.

In the literature data are given on the chemical composition of wild mushrooms (AMKE et al., 1989; CHEIKO & HIROSHI, 1985; GAST et al., 1988; HARYNA & RONDA, 1988; KIKUCHI et al., 1984; KURASAWA et al., 1982; NAGY, 1987, 1988; SAWAYA and co-workers, 1985; SEEGER and co-workers, 1983; SUORTTI, 1984; ZAKHARY et al., 1983). Only a few papers have recently appeared on the vitamin composition of wild mushrooms (DUDA et al., 1980; LASOTA & FLORCZAK, 1983; SOUCI et al., 1967). On the other hand, the data reported by different researchers working on the same kinds of wild edible mushrooms are often presented in different ways, with measurements based on highly variable wet or dry weights, making the comparison of such data difficult.

No published data were found on the chemical composition and nutritive value of *Agaricus bisporus* (the button mushroom) and wild mushrooms grown in Turkey. The purpose of the present paper is to provide data on proximate composition and nutritional characteristics (vitamins, minerals) in some edible wild mushrooms grown in Turkey which may also be common to other countries in the area, from the food chemical viewpoint, and evaluate the contribution of wild mushrooms to the daily intake of these components.

Table 1

Commercial world production of edible mushrooms[a]

Species	Common name	Tonnes per annum
Agaricus bisporus	Champignon	750 000
Lentinus edodes	Shiitake	180 000
Volvariella volvacea	Chinese mushroom	65 000
Flammulina velutipes	Winter mushroom	65 000
Pleurotus spp.	Oyster mushroom	40 000
Pholiota nameko	Winter mushroom	20 000
Auricularia spp.	Ear mushroom	12 000
Tremella spp.	Jelly mushroom	3 000
Other species		9 000

[a] HADAR & DOSORETZ, 1991

1. Materials and methods

1.1. Materials

Fifty samples of two different species of edible wild mushrooms, Kuzu Göbeği (*Morchella esculanta* St. Amans var. rotunda Pers.) and Sütlüce (*Lactarius deliciosus*) Fr.) were collected in February/March 1993 in Western Turkey.

Freshly harvested button mushrooms, *Agaricus bisporus* (Lange) Sing. were obtained from three different growers (A, B, C) in Turkey. The cultural techniques used by growers were traditional. The composts were prepared from horse manure by grower A, broiler chicken manure by grower B, horse manure (70%) and wheat straw (30%) by grower C. The spawn running was mechanically prepared at 0.85% and the plastic bags were filled with 35 kg of compost. The temperature in the mushroom bed was 13–16 °C.

1.2. Proximate analysis

Ash, fat, moisture and protein were determined using the standard methods of A.O.A.C. (1990). Total amount of carbohydrate was estimated by substracting the amount of ash, protein and fat from dry matter. Crude protein was expressed as 4.38×N.

1.3. Vitamin analysis

Methods outlined in A.V.C. (1966), A.O.A.C. (1990), ÖTLES (1992) and STROHECKER and HENNIG (1963) were used for the analysis of water soluble vitamins B_1, B_2, C, folic acid, pantothenic acid and PP.

1.4. Mineral element analysis

For the determination of mineral elements (Zn, Cu, K, Na, Fe, Ca, Cr and P), the ash was dissolved in 5 ml 20% HCl (A.O.A.C., 1990). The final diluted solution for Mg and Ca contained 1% lanthanum to overcome interferences, especially by phosphates. All mineral elements except K, Na and P were determined with an atomic absorption spectrophotometer (Pye Unicam, Model SPB). K and Na were determined with flame emission technics on the same instrument. P was determined spectrophotometrically using the procedure of NAGY (1987).

All the analyses were carried out in three replicates.

2. Results and conclusions

2.1. Proximate compositions

Table 2 contains the summing up of the results of proximate analysis together with mean values regarding the ash, fat, moisture, protein and total sugar contents of cultivated mushrooms (*Agaricus bisporus*). The cultivated mushrooms from three different growers had similar distributions of nutrients in proximate composition. The data in Table 2 obtained in this study are in agreement with those obtained by ANON (1986), MITCHELL and SAVAGE (1990), while values of protein and fat are higher than those reported by KOSSON and BAKOWSKI (1984), KURASAWA and co-workers (1982). It can be seen that *Agaricus bisporus* has very high total dietary fibre content which increases the importance of its role in human nutrition, and which consists of hemicellulose, chitin- and pectinate-like substances, as indicated by the literature (LEVAI, 1989; STANTON, 1984; ZAKHARY, 1983). The relative contribution in meeting the U.S. RDA (Recommended Daily Allowances) indicated that the proteins (Table 2) of *Agaricus bisporus*, which lacks some of the essential amino acids, represent the major components, followed by carbohydrates and ash (mineral elements).

In Table 2, a summary is given of the contents of ash, fat, moisture, protein and total carbohydrates of wild mushrooms grown in Turkey. The average contents were as follows; crude protein: 3.05, crude fat: 0.29, ash: 1.26, total carbohydrates: 5.85, respectively (% in wet basis). Differences were found in total carbohydrates and proteins between Sütlüce and Kuzu Göbeği mushrooms.

Table 2

The proximate composition of Agaricus bisporus *and wild mushrooms*[1]

Compost of grower A: horse manure (100%)
Compost of grower B: Broiler chicken manure (100%)
Compost of grower C: horse manure (70%) and wheat straw (30%)
Mushroom bed temperature: 13–16 °C

Grower/Strain	A	B	C	Mean	RDA (%)	Morchella	Lactarius	RDA (%)
Ash	0.87ab	0.61c	0.79b	0.76b		1.23a	1.29a	
Fat	0.94a	0.44b	0.31bc	0.56ab		0.22c	0.36b	
Moisture	93.57a	92.26a	91.45a	92.43a		86.32b	89.44ab	
Protein[2]	2.72ab	2.51b	1.95c	2.39b	3.7	2.78ab	3.31a	4.7
TDF[3]	0.52c	2.87b	4.43a	2.63b				
Total sugars (Available carbohydrate)	0.22a	0.24a	0.24a	0.23a				
TC[4]	0.74c	3.11b	4.67ab	2.86bc		8.15a	3.55b	

[1] expressed in mg/100 g on wet weight basis

[2] N×4.38

[3] total dietary fibre, the amount was estimated by substracting the amount of ash, fat, protein and total sugars from dry matter

[4] total carbohydrates, the amount was estimated by substracting the amount of ash, fat and protein from dry matter

a–c means with different superscript within the same column are significantly ($p<0.05$) different

RDA%: Percentages of U.S. RDA (Recommended Dietary Allowances)

The contents of other components in proximate composition of wild mushrooms were very nearly the same. Our results for *Lactarius* and *Morchella* strains obtained in this work are in good accordance with the data in the literature, while the values for protein are higher than those reported by KURASAWA and co-workers (1982), SOUCI and co-workers (1967). The data of the relative contribution in meeting the U.S. RDA of proteins, wild mushrooms are 7.3% for *Lactarius* strain and 6.1% for *Morchella* strain, respectively.

2.2. *Vitamin compositions*

Table 3 shows the results of vitamin analyses together with mean values regarding the vitamin B_1, B_2, C, folic acid, pantothenic acid and PP contents of *Agaricus bisporus*. The vitamin contents of *Agaricus bisporus* from growers B and C were very nearly the same. The mushroom samples from grower A showed somewhat greater contents of vitamin C and PP, but their vitamin B_2 contents were lower than in samples from other growers. The results for vitamin contents are in close agreement

with those reported in literature (ANON, 1986). DUDA and co-workers (1980) reported that the *Agaricus bisporus* had 0.021 mg/100 g vitamin B_1, 0.263 mg/100 g vitamin B_2, 12.4 mg/100 g vitamin C and 4.3 mg/100 g vitamin PP. The vitamin B_1 and PP values reported by them were less than those obtained by us, while the vitamin B_2 and C contents were higher. It seemed that the relative contribution in meeting the U.S. RDA showed that *Agaricus bisporus* is a good source of vitamins, especially the B complex vitamins, riboflavin (vitamin B_2), folic acid, pantothenic acid and niacin (vitamin PP), as indicated by the results presented in Table 3.

Table 3

The vitamin composition of Agaricus bisporus *and wild mushrooms*[1]

Compost of grower A: horse manure (100%)
Compost of grower B: Broiler chicken manure (100%)
Compost of grower C: horse manure (70%) and wheat straw (30%)
Mushroom bed temperature: 13–16 °C

Grower/Strain	A	B	C	Mean	RDA (%)	Morchella	Lactarius	RDA (%)
B1	0.075b	0.088b	0.069bc	0.077b	5.1	0.144a	0.118ab	8.7
B2	0.364b	0.472a	0.415ab	0.417ab	24.5	0.213bc	0.103c	9.3
C	6.12ab	5.25bc	5.43b	5.60b	9.3	7.36a	5.22bc	10.5
Folic acid	0.065ab	0.055b	0.055b	0.058b	14.5	0.075a	0.036bc	13.9
PA	1.94c	2.35b	1.91c	2.07bc	20.7	3.16a	2.62ab	28.9
PP	5.86a	5.13ab	4.96b	5.32ab	26.6	6.39a	6.15a	31.4

[1] expressed in mg/100 g on wet weight basis
a–c means with different superscript within the same column are significantly ($p<0.05$) different
RDA%: Percentages of U.S. RDA (Recommended Dietary Allowances)
PA: Pantothenic acid

Table 3 shows the results of vitamin analyses with the mean values regarding the vitamins B_1, B_2, C, folic acid, pantothenic acid and PP contents of *Morchella* and *Lactarius* strains. There was considerable variation in the vitamin composition due to type of mushroom. The samples of *Morchella* strain showed somewhat greater contents of vitamins B_1, B_2, C, folic acid, pantothenic acid and PP than the samples of *Lactarius* strain. This is not in agreement with SOUCI and co-workers (1967) who found no differences in the vitamin composition of *Morchella* and *Lactarius* strains. On the other hand, the vitamin contents obtained in this study were higher than those obtained by the above authors. DUDA and co-workers (1980) reported that the *Lactarius deliciosus* had 0.054 mg/100 g vitamin B_1, 0.298 mg vitamin B_2, 2.83 mg vitamin C and 3.6 mg vitamin PP. The vitamin B_1, C and PP values reported by them

were less than those obtained by us, while the vitamin B_2 content was higher. As it can be seen from Table 3, the relative contribution in meeting the U.S. RDA (Recommended Daily Allowances) showed that, among the water soluble vitamins, vitamin B_2 (riboflavin), C (ascorbic acid), folic acid, pantothenic acid and PP (niacin) are at high levels in wild mushrooms.

2.3. Mineral element compositions

Table 4 compares the mineral element composition of *Agaricus bisporus* samples obtained from three different mushroom growers and gives the mean values. The concentration of potassium (important to balance the sodium in salt) was the highest in all the mushroom samples followed by phosphorus (for strong bones), sodium, zinc and calcium. Major differences were observed in the levels of these macroelements in the mushrooms obtained from three different growers. The data obtained in this study (excl. zinc) are in agreement with those reported by ANON (1986), KIKUCHI and co-workers (1984), SCHINDLER (1985), SEEGER and co-workers (1983), STANTON (1984). Part of the dietary problem with Na is the imbalance between K and Na. As foods become more processed, the potassium content decreases, while the sodium content increases (STANTON, 1984). As it can be seen from Table 4, *Agaricus bisporus* samples in our study had plenty of potassium and were very low in sodium when compared to daily needs (Table 4). On the other hand, the zinc content was several times higher than that reported by the above authors. Among the microelements, iron (needed to prevent anaemia) and copper were present in higher concentration while chromium was present in trace amounts when compared to daily needs. The results for microelements of *Agaricus bisporus* obtained in the present work are in agreement with those reported by the above authors.

The mineral element composition of *Morchella* (Kuzu Göbeği) and *Lactarius* (Sütlüce) strains and mean values are given in Table 4. The concentration of potassium was the highest in both mushroom strains followed by phosphorus, sodium, iron and calcium. Major differences were observed in the levels of macroelements in *Morchella* and *Lactarius* strains. The results in Table 4 are generally in agreement with those reported by GAST and co-workers (1988), KIKUCHI and co-workers (1984), NAGY (1987 and 1988), SEEGER and co-workers (1983), SOUCI and co-workers (1967). *Morchella* and *Lactarius* mushrooms in our work had plenty of iron and were very low in calcium when compared to daily needs (Table 4). Among the microelements, iron and copper were present in high concentration, while chromium was present in trace amounts when compared to daily needs, as demonstrated by the results presented in Table 4. The results for microelements of wild mushrooms obtained by us are in agreement with those reported by the above authors.

Table 4

The mineral elements of Agaricus bisporus *and wild mushrooms*[1]

Compost of grower A: horse manure (100%)
Compost of grower B: Broiler chicken manure (100%)
Compost of grower C: horse manure (70%) and wheat straw (30%)
Mushroom bed temperature: 13–16 °C

Grower/Strain	A	B	C	Mean	RDA (%)	Morchella	Lactarius	RDA (%)
Ca	31.39b	32.47b	27.50b	30.45b	3.0	46.15ab	81.77a	12.8
Cr	trace	trace	trace	trace	trace	trace	trace	trace
Cu	5.50b	8.53a	7.27ab	7.10ab	355.0	3.34bc	5.29b	215.8
Fe	8.88b	6.96b	7.87b	7.90b	43.9	61.57ab	89.12a	418.6
K	1250.0c	1751.0b	1539.8bc	1513.6bc	80.7	3398.0a	2764.0ab	164.3
Na	67.50b	52.33b	30.00b	49.94b	4.5	432.0ab	508.7a	42.8
P	1086.9ab	995.2ab	687.5bc	923.2b	92.3	1658.9a	1604.3a	163.2
Zn	41.26a	42.50a	39.93a	41.23a	274.9	12.62ab	7.81b	68.1

[1] expressed in ppm
a–c means with different superscript within the same column are significantly ($p<0.05$) different
RDA%: Percentages of U.S. RDA (Recommended Dietary Allowances)

*

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A RESEARCH NOTE ON SOME PROCESS CONDITIONS OF ONION RING DRYING

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In this paper, the drying behaviour of onion slices (*Allium cepa*) was investigated from technological and microbiological standpoints. The influence of process conditions, such as type of drying system, air temperature and onion slice thickness were studied on the specific drying rates and microbial loads. Trials were carried out using a pilot plant (air-flow drier) and on a cabinet drier. Water content, water activity, specific drying rates and microbial load of the intermediate and final products were determined.

Results have shown that, in case of sliced onions, by using the air-flow drying system, higher dehydration rates and lower microbial loads can be achieved compared to standard cabinet driers.

Keywords: onion rings, drying, hygienic state, air-flow drier

Dehydrated onions have become a standard ingredient in several foods. This product is commonly used by industries for the preparation of foods such as ketchup, chili sauce, meats and sausages, crackers and snacks. Food services frequently use dehydrated onions (rings and powder) because of the easiness in food preparations and storage (SILBERSTEIN et al., 1984; DALL'AGLIO et al., 1986). In fact, processed onions have several advantages over the "natural product"; the dried onions are not subjected to seasonal fluctuations, are more reproducible in organoleptical quality and moreover, they are easily dispersed in foods (FENWICK & HANLEY, 1986). Dehydrated onions are normally processed with air-flow driers until a low water content of 0.04–0.05 g H_2O/g dry matter is reached; however, the safe water content, as lower limit for fungal growth, is about 0.19 g H_2O/g dry matter, corresponding to a water activity value of about 0.7 (FENWICK & HANLEY, 1986).

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As it is well known, the quality of dehydrated onions is widely influenced by the characteristics of the raw material such as chemical composition, texture and microbial load, by drying technologies and process parameters (CARPI & DALL'AGLIO, 1988; LERICI, 1985; DALL'AGLIO et al., 1987); the microbial load and the types of microorganisms are the result of interaction of various factors during the processing of products (SHENEMAN, 1973; VAUGHN, 1970; BARBANTI et al., 1991). These include the high degree of contamination of the raw onions by the soil, the lack of an effective bactericidal heat treatment and the dehydration method (SILBERSTEIN et al., 1984; FIRSTENBERG et al., 1991).

In this paper, we report the results of some drying trials carried out on onion rings.

The difference of specific drying kinetics between air-flow and cabinet drier were evaluated. The influence of some drying parameters (air temperature and onion slice thickness) on the specific drying rates and on the microbial load of onions were quantified using only the air-flow drier. Water content, water activity, specific drying rates and microbial load of the intermediate and final products were measured.

1. Materials and methods

Onion samples (*Allium cepa*, cv. Dorata of Bologna) were manually washed, peeled and cut in slices of 4 and 8 mm in order to obtain two sample groups.

The drying equipments used in our experiments were:

- air-flow drier (Sandvik Process Systems, Milan, Italy), as described in previous papers (BARBANTI et al., 1994; BARBANTI et al., 1995). The air properties were: air temperature (at ambient conditions) = 20 °C; air absolute humidity = 0.012 kg H_2O/kg air; the air velocity inside the equipment was set at 1.5 m/s; the product load was of 24 kg m^{-2}, corresponding to an initial product thickness (for 8 mm slices) of 16 cm. Temperatures inside the air drier and at the centre of the product load were measured through thermocouples connected to a digital two-channel thermometer (Crison Instruments, Modena, Italy).

In this pilot plant, three different combinations of time and temperature were chosen for drying sliced onions: A: 115 °C, 10 min; 90 °C, 20 min; 80 °C, 30 min; 70 °C, 30 min; 60 °C to end process, considered at a product water content = 0.06 g H_2O/g dry matter; B: 70 °C throughout the process; C: 130 °C, 10 min; 100 °C, 20 min; 90 °C, 30 min; 80 °C, 30 min; 60 °C to end process.

- cabinet drier (Vismara model STF, Milan, Italy), without air forced circulation; the total product load was of 24 kg m^{-2} (4 trays, 6 kg m^{-2}) and product thickness was (for 8 mm slices) of 12 cm. The drying temperature was 70 °C for all the process.

In order to evaluate the variability of the drying trials, three repetitions in the air-flow drier at 70 °C of onion rings of 8 mm thickness were carried out; the average value of coefficient of variation was of 3.7%; this value was considered acceptable, keeping into account the aim of the research note. Data reported are average values of the replicates.

1.1. Analytical determinations

The water content of onion rings was determined after drying in a vacuum oven for 8 h at 70 °C, following the official method of analysis (A.O.A.C. 1984).

Water activity of onions was determined after 24 h of sample equilibration (IGLESIAS & CHIRIFE, 1982); an electric hygrometer (Rotronic Hygroskop BT, Zurich, Switzerland) thermostated at constant temperature (20 °C) was used. The instrument was previously calibrated with the following saturated salt solutions: LiCl (a_w=0.115); $MgCl_2$ (a_w=0.327); $Mg(NO_3)_2$ (a_w=0.552); NaCl (a_w=0.758); KCl (a_w=0.844). By plotting experimental versus theoretical a_w data a regression line with equation $Y=0.999X-0.003$ and r^2 value of 0.999 was obtained.

For the microbial count, opportune decimal dilutions of samples to be analyzed were plated on Plate Count Agar (PCA, Difco) in order to obtain the mesophilic aerobic count, and on Violet Red Bile Agar (VRBA, Difco) for total and fecal coliform counts. The dishes were incubated at 30, 37 and 44 °C in thermostated cells, respectively, for mesophilic aerobic count, total and fecal coliforms (SHENEMAN, 1973).

All analyses were performed in triplicate for each onion sample; from the average value and the standard deviation of each group of three repeated analyses, were then calculated the coefficient of variations (CV). The average CV values were 3.7 for water content determination (A.O.A.C. 1984), 2.2 for a_w determination and 11.5% for microbial count.

2. Results

During drying, air and onion sample temperatures were continuously measured with two thermocouples connected to a digital two-channel thermometer and recorded on an XY chart plotter. As an example, the temperatures of air and an onion sample, dried in air-flow drier, using the time-temperature combination C, are shown in Fig. 1. As it can be seen, the temperature of the product, because of the latent heat of water evaporation, is always below 90 °C and tends to the air temperature value after 60 min these temperature values, reached in the centre of the product load, did not cause non-enzymatic browning of onion samples.

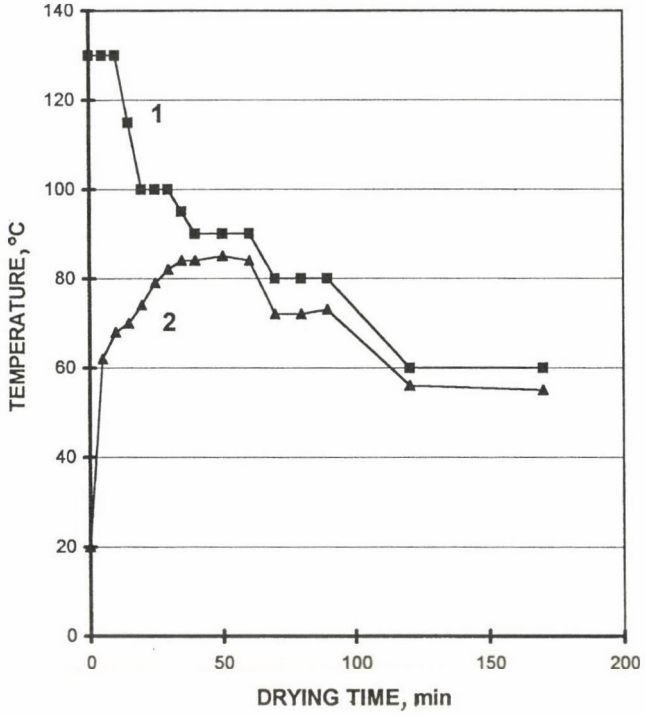

Fig. 1. Temperature profiles of air inside the air-flow drier and at the centre of the product load measured during a drying trial with time–temperature combination C. 1: air temperature inside the drier; 2: air temperature at the centre of the product load

The first drying trial has been carried out comparatively on the two different drying equipment (air-flow and cabinet driers) at constant temperature (70 °C), using onion rings of 8 mm thickness; the results are shown in Fig. 2. As expected, the cabinet drier was the slower system to dry onion slices; moreover, it was observed that after 270 min, onion samples were still not completely dried. Under these process conditions, onion drying carried out in air-flow drier had better results, both in terms of drying time and product quality. Hence, in order to test the influence of air temperature and thickness of onion slices on the specific drying kinetics and rates of samples, further drying trials were carried out with the air-flow drier only.

In Fig. 3 the specific drying rates (g H_2O/g dry matter min$\times$1000) are reported as a function of water content (g H_2O/g dry matter) of onions of 8 mm thickness dried with air-flow pilot plant using time-temperature combinations A, B and C. Dehydration at high temperature (C, starting temperature = 130 °C), under our experimental conditions, gave as a result high values for specific drying rate (about

11500 g H_2O/g dry matter min×1000), while the cycle A (starting temperature = 115 °C) and cycle B (constant temperature = 70 °C) showed initial values of drying rate of 8000 and 4000 g H_2O/g dry matter min, respectively.

During air drying of vegetables, the reduction of product thickness leads to a consequent decrease of the drying time. In particular, by reducing the onion ring thickness from 8 to 4 mm the time needed to reach a final water content of about 0.25 g H_2O/g dry matter decreased from 330 min (8 mm) to 120 min (4 mm) as reported in Fig. 4; this trial was carried out on the air-flow drier with air temperature cycle A.

Plotting water content values of the product during process against the corresponding water activity values, at constant temperature cycle of 70 °C, the desorption isotherm curve can be obtained (Fig. 5). The highest value plotted on the graph (3.75 g H_2O/g dry matter, a_w=0.960), was measured after 100 min of drying time; the lowest one (0.1 g H_2O/g dry matter, a_w=0.305) was measured after 450 min of drying. These data are in accordance with those reported by IGLESIAS and CHIRIFE (1982).

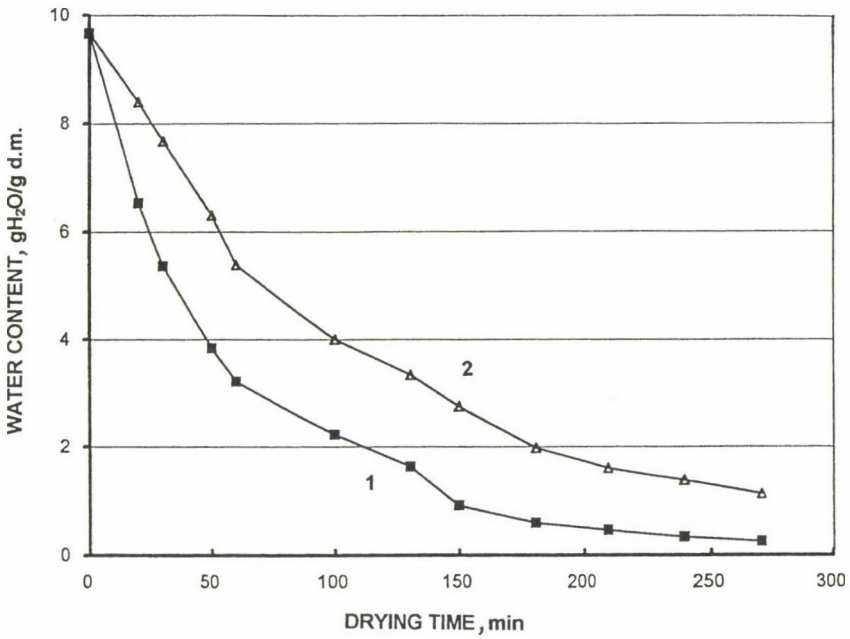

Fig. 2. Specific drying kinetics, expressed as water content (g H_2O/g dry matter), of onion rings as a function of the drying time for air-flow and cabinet driers. Temperature of drying: 70 °C for all the length of process. 1: air-flow drier; 2: cabinet drier

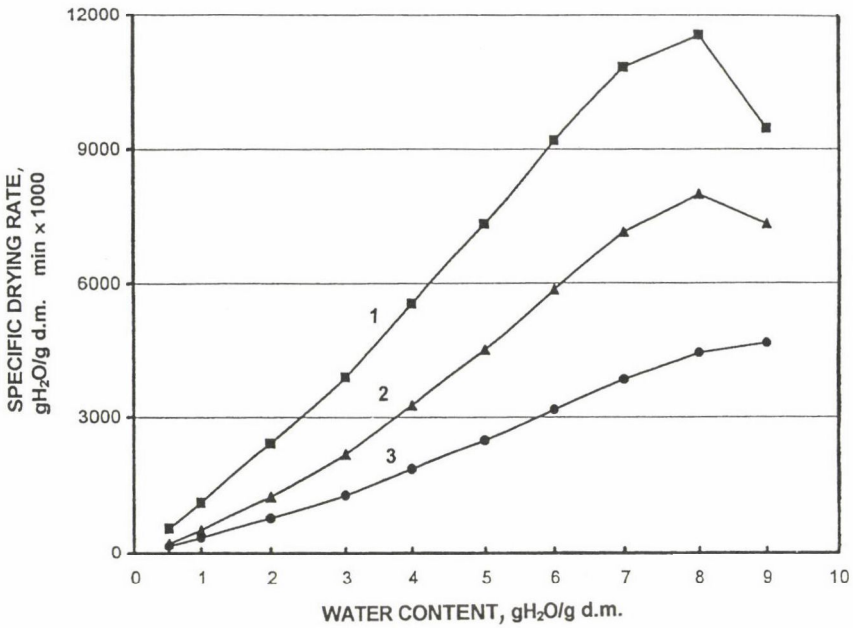

Fig. 3. Specific drying rates (g H_2O/g dry matter min) as a function of water content (g H_2O/g dry matter) for time–temperature combinations A, B and C, obtained with air-flow drier. 1: C; 2: A; 3: B

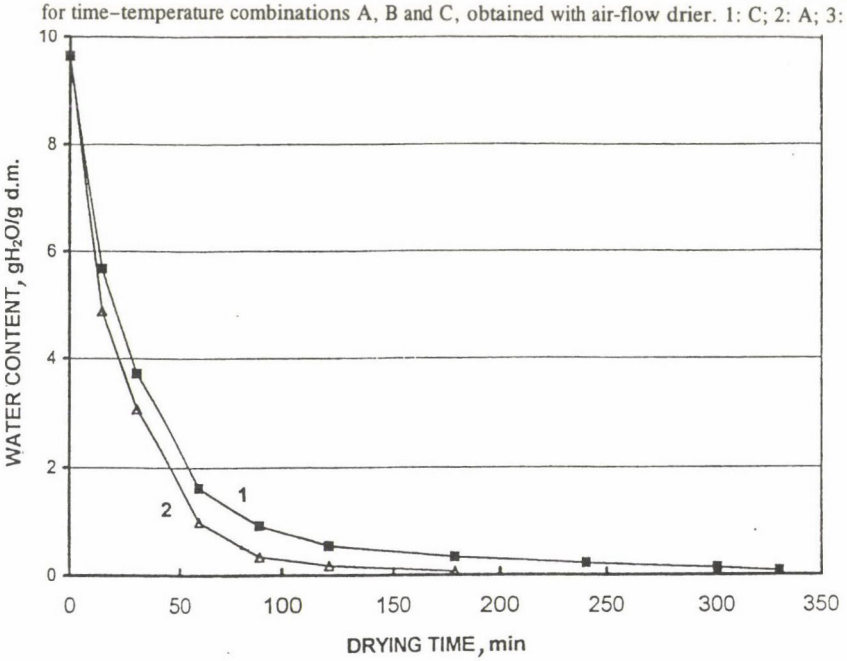

Fig. 4. Specific drying kinetics obtained using time–temperature combination A for onion rings of 4 and 8 mm thickness, carried out with air-flow drier. 1: 8 mm thickness; 2: 4 mm thickness

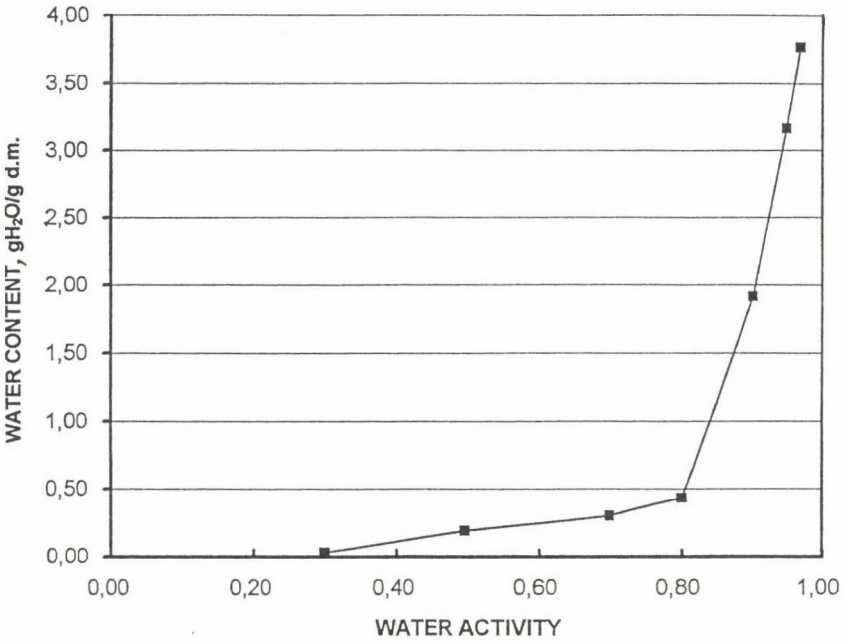

Fig. 5. Desorption isotherm curve for 8 mm thickness onion rings obtained at constant temperature of 70 °C (time–temperature combination B)

In Table 1 microbial counts of raw, intermediate and final products (slices of 8 mm) are summarized. The drying trials carried out with air-flow equipment (time--temperature combinations A, B and C) strongly reduced the mesophilic aerobic count, and it was always lower than 1.84 log CFU/g, the total and fecal coliforms were always below the detection level (< 1 log CFU/g); moreover, as expected, the process temperatures influenced the microbial load at the intermediate phase of the drying cycle. On the other hand, the drying experiments carried out with cabinet drier, while reduced the fecal coliforms number below 1 log CFU/g, were characterized by relatively high values of total mesophilic aerobic count and total coliforms. Data shown in Table 1 revealed that the drying cycles conducted with cabinet drier gave a final product with microbiological quality lower than those obtained by the air-flow one.

Table 1

Microbial count expressed as log of colony forming units/g (CFU/g) for fresh, intermediate and completely dried onion slices, as a function of different drying plants and time temperature combinations

Drying plant	Time – temp. combination	Mesophilic aerobic count (log CFU/g)		Total coliforms (log CFU/g)		Fecal coliforms (log CFU/g)	
		fresh onions = 6.81		fresh onions = 4.16		fresh onions = 3.75	
		intermediate	dried	intermediate	dried	intermediate	dried
air-flow	A	2.77	1.38	<1.00	<1.00	<1.00	<1.00
	B	4.78	1.86	<1.00	<1.00	<1.00	<1.00
	C	2.00	1.32	<1.00	<1.00	<1.00	<1.00
cabinet	B	5.32	3.25	3.80	3.10	<1.00	<1.00

3. Conclusions

The results of these experiments on onion drying gave evidence of the efficiency of air-flow drier, not only in terms of process rates, but also regarding the general quality characteristics of the final products. In particular, from a microbiological standpoint, it was observed that samples dried in air-flow equipment were subjected to a strong reduction of the microbial load.

As expected, in case of static cabinet drier, long drying times (>450 min) and relatively high microbial loads were measured.

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IMMOBILIZATION OF LIPASE AND ITS INVESTIGATION

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The enzymatic transformation of food industrial raw materials such as fats and oils, has been carried out with the use of immobilized lipases. This way the value of lower quality raw materials can be improved. First step of the bioconversion method is the immobilization of lipase. Lipase preparations of different origin (*Rhizopus sp.*, porcine pancreas and *Penicillium roqueforti*) were immobilized on synthetic polymers (DUOLITE resin, PVC), on glass-based carrier (AP-685, ALD-610) and on aluminium oxide carrier. The conditions of the immobilization were studied in aqueous and hexaneous solvents, at various temperatures (20–60 °C) and pH-values (7.0–9.0).

Immobilized lipase preparation with the greatest lipase activity was examined in column reactor, measuring the diminution of enzyme activity as a function of time. Following a 48 hours' reaction period, the relative activity decreased somewhat below 10%. Using immobilized lipase the bioconversional process can be regulated. From industrial waste fats and oils valuable raw material can be produced.

Keywords: immobilization; lipase, E.C.3.1.1.3.; DUOLITE ES 568, PVC, glass-based and aluminium oxide supports

The immobilization of enzymes is a basic method of their use in the industry. One domain of the enzyme transformation of food industrial raw materials is the special use of lipase for the decomposition of fats and oils. The lipase immobilization demands careful pre-trials. The selection of suitable carrier, the problems of immobilization and industrial conditions, the reaction mechanisms, the diluting agents, the interfacial problems of liquid and solid phases have all to be considered (BÁNKY, 1990; TEMESVÁRI et al., 1994).

1. Materials and methods

1.1. Enzymes and carriers

In our immobilization trials, lipase preparations from Rhizopus sp. 64 U mg^{-1}, porcine pancreas 20 U mg^{-1} (lyophilized SERVA) and *Penicillium roqueforti* 30 U cm^{-3} (KÉKI laboratory culture) were applied.

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As enzyme carriers, DUOLITE ES 568 ion exchange resin, PVC synthetic material smaller than 100 μm, glass-based AP-685, ALD-610 and AP-aluminium oxide materials were used. Immobilization was carried out in aqueous and hexanous buffer solvent, as follows.

1.2. Immobilization methods

1.2.1. Covalent immobilization in aqueous medium. Ten g dry carrier was shaken at 20–60 °C temperature at ten degree intervals, in 50 cm^3 pH=6–8, 0.05 mol phosphate buffer in a Vibrotherm apparatus. Then 1 g lipase dissolved in 50 cm^3 phosphate buffer was added.

The enzyme with the carrier was left to react at the same temperature for 20 h. After this, shaking was continued for another 30 min., in 1% glutaraldehyde solution. After draining on filter, it was washed at 4 °C with buffer, drained to dryness, then stored at 4 °C until further use.

1.2.2. Adsorptive immobilization in aqueous medium. We applied the aqueous covalent immobilization method, except for the treatment with glutaraldehyde.

1.2.3. Covalent immobilization in hexane medium. For solvent immobilization 90% *n*-hexane buffer solution was used and the method was the same as in the previous method (1.2.2.).

1.2.4. Adsorptive immobilization in hexane solvent medium. It was similar to the aqueous adsorptive immobilization method except that the buffer was stirred with 90% *n*-hexane during fixation. The enzyme was immobilized with icy *n*-hexane.

1.3. Assay procedure

The result of immobilization was controlled by measuring the lipase activity of the product.

Lipase activity was measured at 410 nm (Pye Unicam SP6–550 UV/VIS Spectrophotometer). The reaction cuvette contained in a final volume of 3 cm^3 50 mmol phosphate buffer, 3 mmol p-NPP (p-nitro-phenyl-palmitate), and the appropriate amount of enzyme. The reference solution contained the complete reaction mixture without enzyme, and p-NPP was added simultaneously to both cuvettes.

The lipase activity was determined in 50 mmol phosphate buffer at pH 8.0, incubated at 37 °C for one hour. Lipase activity is proportioned to the absorbance value 1.0 A^{410}=9.7 μmol p-NPP (BARISZLOVICH et al., 1990).

One unit of lipase activity (U) was defined as the amount of lipase which catalyzed the production of 1 μmol of p-nitrophenol per hour under the indicate experimental conditions. The method proved to be well reproducible, the standard deviation of parallel measurements being 3–5%.

2. Results

2.1. Effect of temperature and pH on the activity of lipase preparations

During measurement of lipase activity, the lipases of various origin often caused surprises. It was important for this reason, to determine the relative activities of the three lipase enzymes between pH-values 7–9 and at 20–60 °C (Table 1).

Based on the determination it could be stated, that all three enzymes could be measured at pH 8.0 and at 40 °C (BARISZLOVICH et al., 1990). Activity measurements made with the test samples were taken at these values.

2.2. Production of immobilized lipase

Three lipase preparations of various origins were immobilized onto five carriers, in aqueous, hexanous solvents, by adsorptive and covalent attachment methods. The activity of the lipase of Rhizopus origin, marked with "R", was 68 U mg^{-1}, 1 g of this was put into reaction in the immobilization operations with 10 g carrier (6800 U g^{-1} carrier). Original concentration values of the lyophilized dry product with marking "P" made of porcine pancreas are the followings: 3 g enzyme with an activity of 20 U mg^{-1} carrier). With the laboratory preparation of the KÉKI marked "S", using 1000 cm^3 enzyme solution, 3000 U g^{-1} carrier activity value could be produced. The original activity of the KÉKI "S" enzyme solution could not be increased, other operations such as lyophilization, chromatographic concentration would have been necessary for further purification and this was not justified for our trials.

To select the optimal conditions of immobilization, pH value was varied between 6–9 and the temperature between 20–60 °C.

2.3. Effect of pH on immobilized lipase activity

Three lipase products were immobilized at a constant temperature of 40 °C and between pH values 6–9. The pH value was adjusted with phosphate buffer solution. On Figures 1–5, the activity of enzymes covalently bound to the carrier in hexanous medium are presented as a function of pH an expressed as relative activity (number of data, n=5). Activity is calculated as percentage and the maximum activity was taken as 100% in the given trial series. The activity curves were similar in tendency regarding to the immobilization medium and method (aqueous or hexaneous, covalent or adsorptive). In all fixing variations, the maximum lipase activity was measured at pH 8.0. The immobilized and free solution pH/activity profiles for the lipase are not very different (Table 1 and Figs. 1–5).

Table 1

Activity of lipase preparations from Rhizopus *sp., porcine pancreas and* Penicillium roqueforti *at different pH and temperature values*

Enzyme activity of lipase preparations						
	Rhizopus sp.		Pancreas		*Penicillium roqueforti*	
at 40 °C pH	U mg^{-1}	%	U mg^{-1}	%	U cm^{-3}	%
7.0	52.4	77	5.0	25	21.3	71
7.5	62.6	92	15.0	75	25.8	86
8.0	68.0	100	20.0	100	30.0	100
8.5	64.6	95	18.0	90	12.9	43
9.0	42.2	62	10.0	50	4.2	14
at pH 8.0 temperature (°C)						
20	68.0	100	20.0	100	30.0	100
30	66.6	98	20.0	100	30.0	100
40	68.0	100	20.0	100	30.0	100
50	46.9	69	15.0	75	23.4	78
60	21.1	31	10.0	50	18.9	63

The lipase activity was measured at pH 8.0, 40 °C, with p-NPP at 410 nm

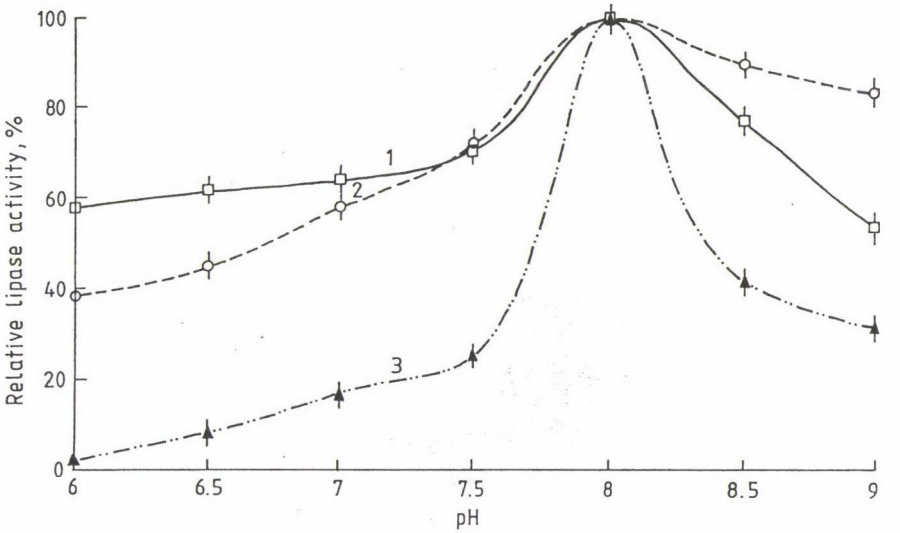

Fig. 1. Effect of pH on immobilized lipase. 1: R, *Rhizopus* sp.; 2: P, porcine pancreas; 3: S, *Penicillium roqueforti* lipases immobilized on Duolite ES 568 carrier, with covalent binding method, at 40 °C, in 50 mmol phosphate buffer pH 6.0–9.0, in 90% (v/v) n-hexane solution. Immob. lipase activity was assayed with p-NPP (See 1.3. Assay procedure)

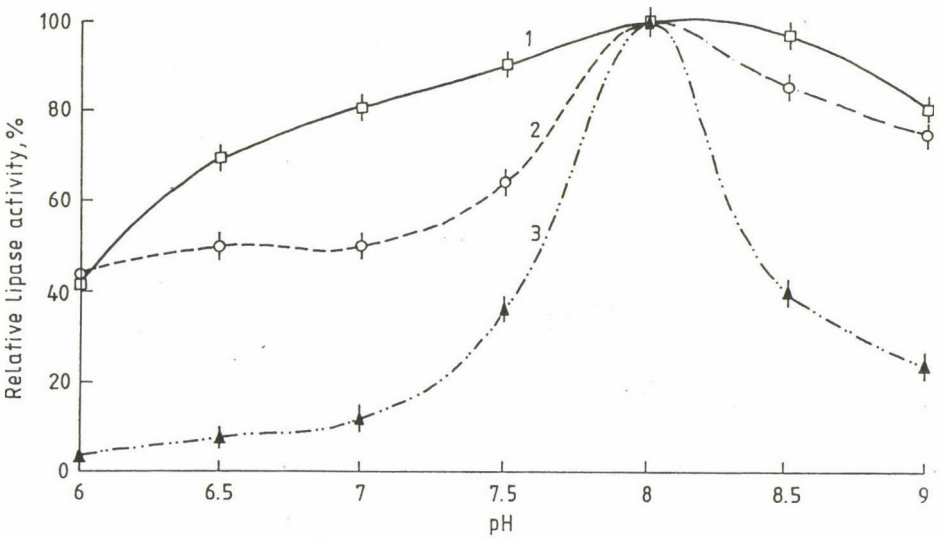

Fig. 2. Effect of pH on immobilized lipase. 1: R; 2: P; 3: S. Carrier: Polyvinyl chloride

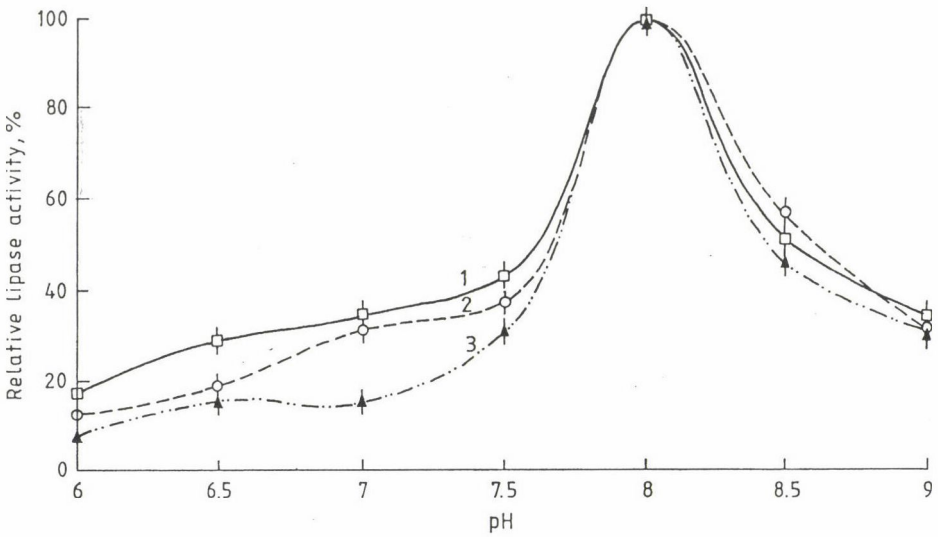

Fig. 3. Effect of pH on immobilized lipase. Immobilization on glass AP–685 carrier. 1: R; 2: P; 3: S

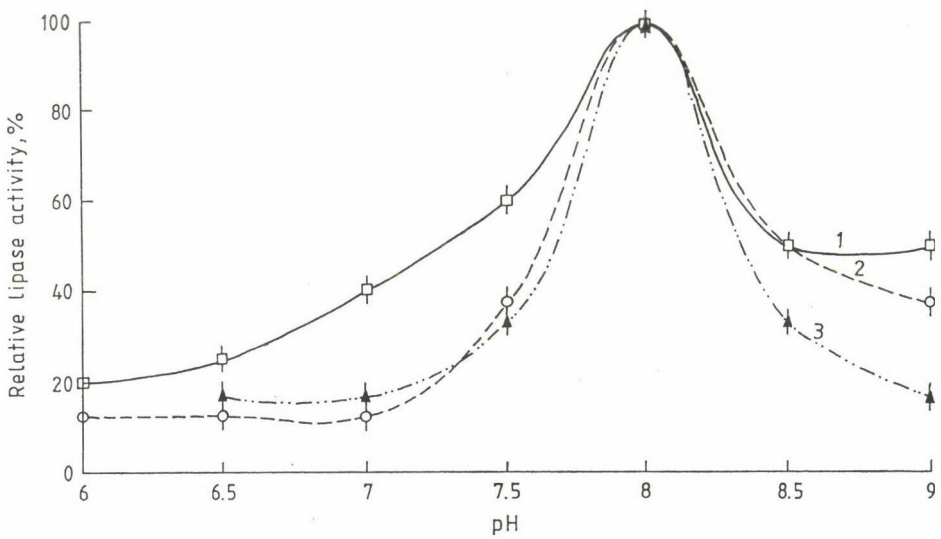

Fig. 4. Effect of pH on immobilized lipase. Immobilization on glass ALD–610 carrier. 1: R; 2: P; 3: S

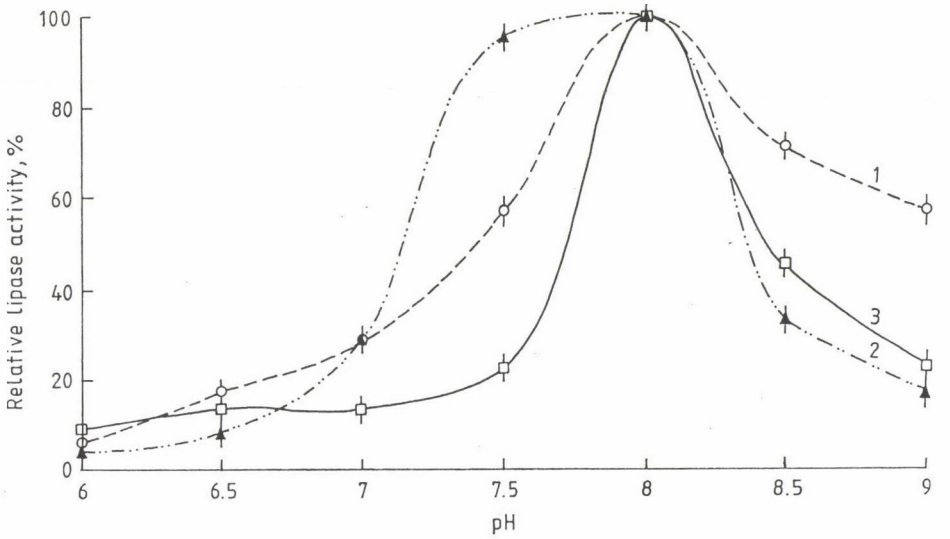

Fig. 5. Effect of pH on immobilized lipase. Immobilization on AP-aluminium oxide carrier. 1: R; 2: P; 3: S

Considering the differences between the carriers, DUOLITE ES 568 and PVC oligomer are capable to fix the enzyme in a greater pH interval than the glass-based and aluminiumoxide carriers. Activity curves in Figs. 3, 4, 5 show steeper peaks near to pH 8.0 than those on Figs. 1, 2. The glass surface of ALD-610 has been prepared for covalent binding, this was proved by the fact, than the glutaraldehyde treatment was equal to the absorption method. No difference was observed between the two methods of immobilization on activity values in case of the carrier ALD-610.

2.4. Heat stability of immobilized lipase

In Figs. 6–10, the changes of immobilized lipase activity as a function of temperature on five carriers are shown. Now, pH value was fixed at pH 8.0 in the immobilization treatments. The Figs. 6–10 show the relative activities measured in covalent aqueous medium. Temperature was changed by 10 degrees between 20–60 °C.

On carriers DUOLITE ES 568 and PVC (Figs. 6, 7), the immobilization of lipase enzymes of different origin is similar at 20 °C and 40 °C, however, the degree of binding is strongly reduced above 40 °C.

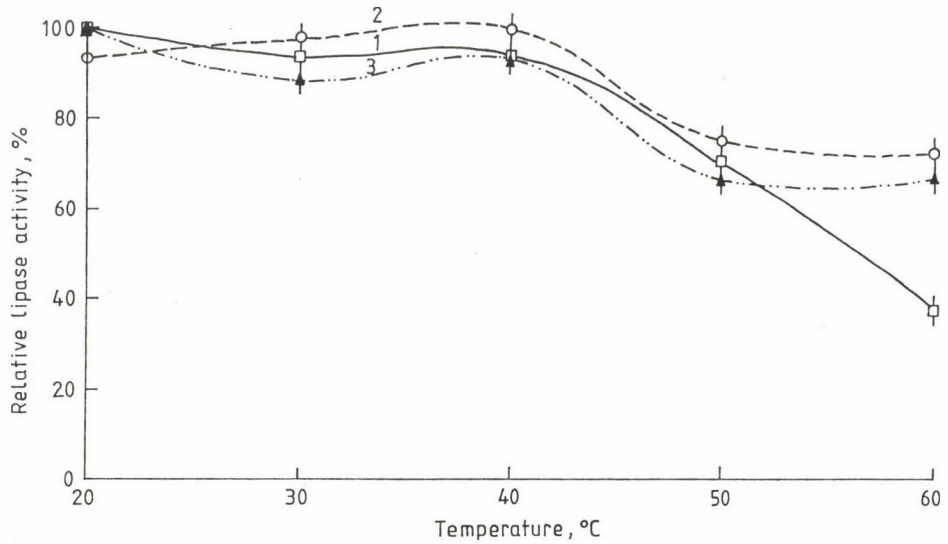

Fig. 6. Effect of temperature on the activity of immobilized lipase. Immobilization was made on Duolite ES 568 carrier, with covalent binding method, at pH 8.0, in 50 mmol phosphate buffer. Enzyme activity was assayed with p-NPP, as described in 1.3. Assay procedure. One hundred percent activity corresponded to the maximum activity of immobilized lipase. 1: R; 2: P; 3: S

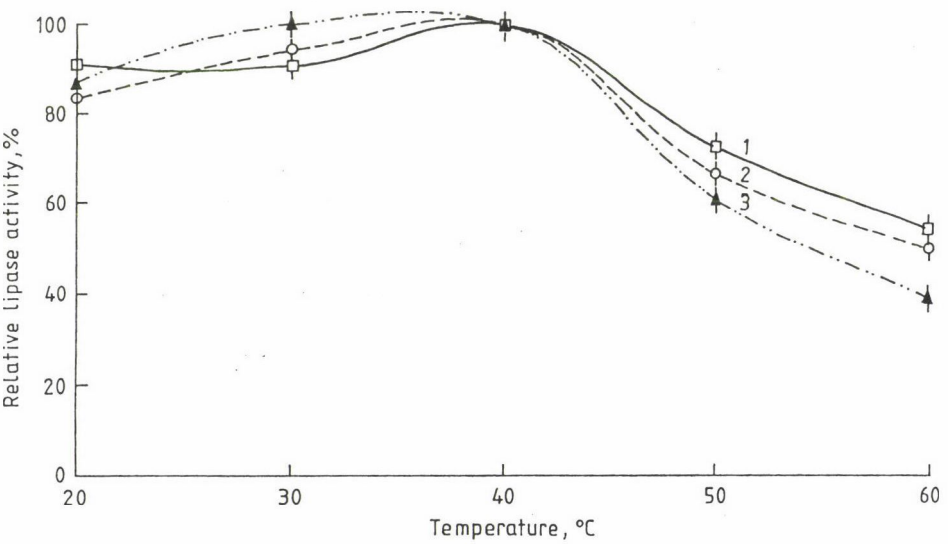

Fig. 7. Effect of temperature on the activity of immobilized lipase. 1: R; 2: P; 3: S. Immobilization on Polyvinyl chloride carrier

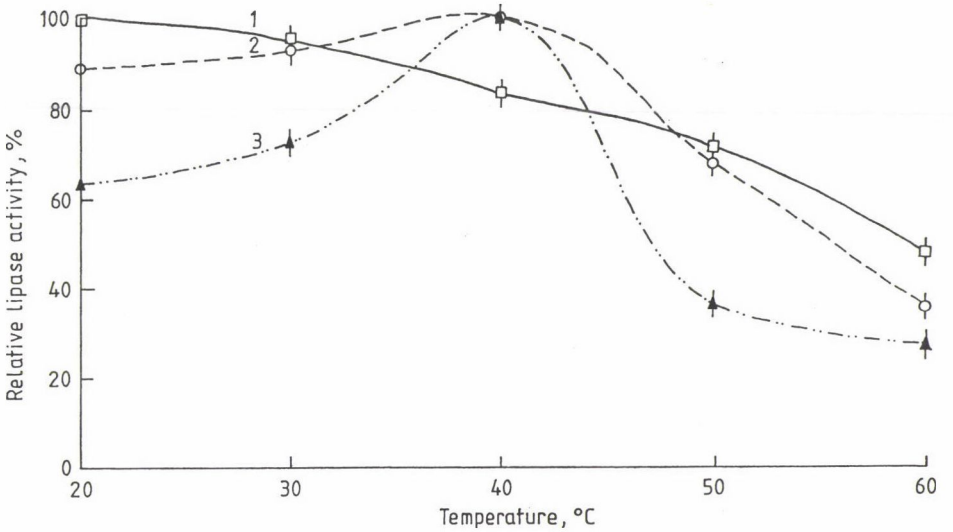

Fig. 8. Effect of temperature on the activity of immobilized lipase. 1: R; 2: P; 3: S. Immobilization on glass AP-685 carrier

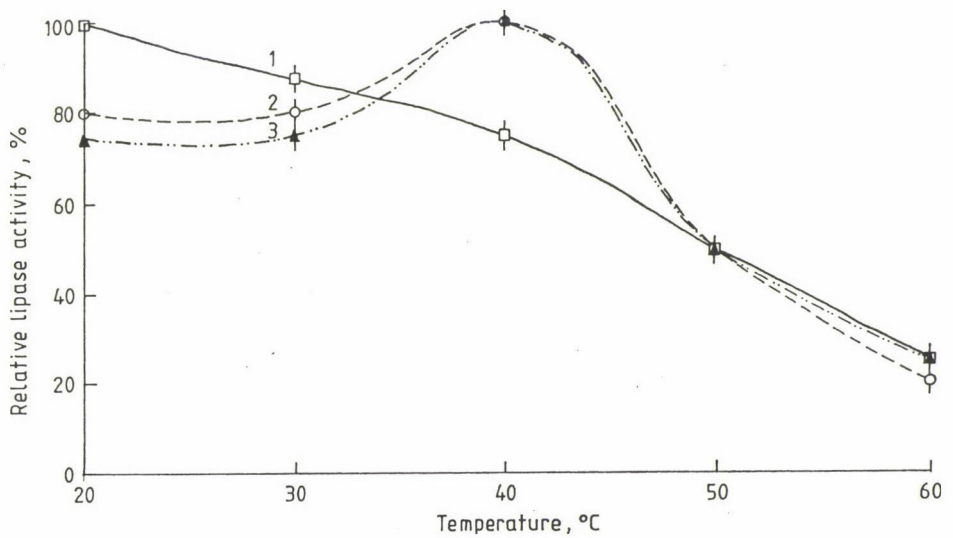

Fig. 9. Effect of temperature on the activity of immobilized lipase. 1: R; 2: P; 3: S. Immobilization on glass ALD-610 carrier

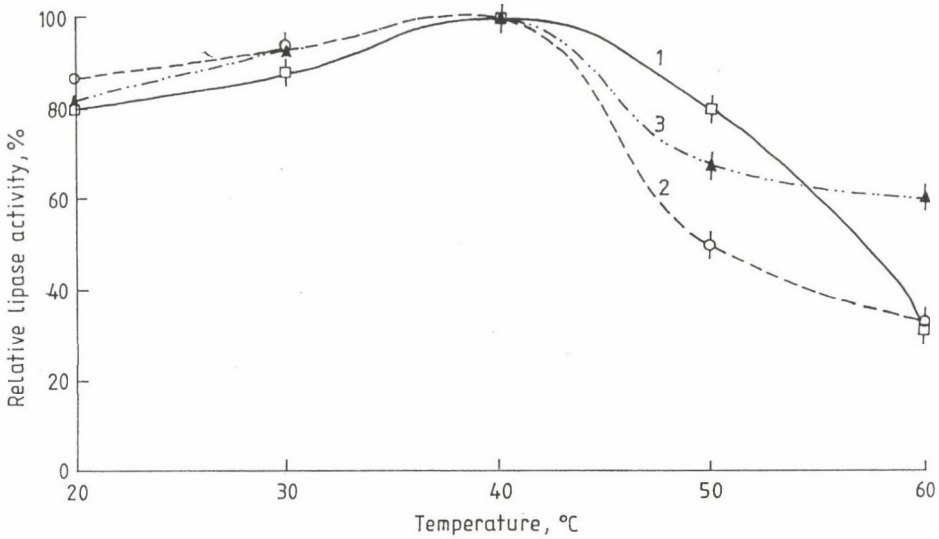

Fig. 10. Effect of temperature on the activity of immobilized lipase. 1: R; 2: P; 3: S. The support: AP-aluminium oxid

On glass based and aluminiumoxide carriers, the changes in activity showed greater fluctuations, than on plastics. The difference in relative activity is significant (P=95%) between lipase preparations of various origin in case of adsorptive immobilization on AP-Al_2O_3 carrier in aqueous phase (Fig. 10), measured at 60 °C.

2.5. Efficiency of the lipase immobilization

Regarding the effectiveness of lipase immobilization, the optimal conditions were found to be at 20–40 °C temperature, with holding the pH-value at 8.0. In Table 2 the activity values of the different lipases immobilized on five kinds of carriers were presented in U g^{-1} carrier units. Percentage of the integration has been calculated taking the initial value of lipase activity used in the immobilization operations as 100%. The percentages of the integration seem to be low compared to the lipase quantity used. Therefore, the enzyme was used in great excess in the fixation procedure.

Based on lipase activity measured in the immobilization procedures, the values assuring optimal conditions were determined by computer analysis (BOX, 1970; VALKO & VAJDA, 1987).

The maximal lipase activity values with corresponding temperature and pH data have been presented in Table 3. Among these data, the highest activity values were

Table 2

Activity and relative activity of immobilized lipase on various carriers

Carriers	Immobilization procedures	Lipase activity (Ug^{-1} carrier) and relative activity (%)					
		Rhizopus sp. lipase		Pancreas lipase		*Penicillium roqueforti* lipase	
		$U\ g^{-1}$	%	$U\ g^{-1}$	%	$U\ g^{-1}$	%
Lipase preparation	–	6800	100	6000	100	3000	100
Duolite ES 568	Cov., W.	80	1.18	80	1.33	42	1.40
	Cov., H.	78	1.15	78	1.30	48	1.60
PVC	Cov., W.	55	0.81	48	0.80	23	0.77
	Cov., H.	72	1.06	56	0.93	25	0.83
Glass AP-685	Cov., W.	35	0.51	28	0.47	11	0.37
	Cov., H.	35	0.51	32	0.53	13	0.43
Glass Ald-610	Cov., W.	30	0.44	10	0.17	4	0.13
	Cov., H.	20	0.29	8	0.13	6	0.20
AP-Al_2O_3	Cov., W.	25	0.37	30	0.50	28	0.93
	Cov., H.	22	0.32	35	0.58	30	1.00

Standard conditions of immobilization: pH 8.0, 40 °C, 20 h. Covalent immobilization, with water and hexane solution, as described in 1.2. Immobilization method

Table 3

Maximum activity of immobilized lipase preparations

Carriers	Immobilized lipase activity								
	Rhizopus sp. Immobilization			Porcine pancreas Immobilization			Penicillium roqueforti Immobilization		
	Temp. (°C)	Procedure	Activity ($U\ g^{-1}$)	Temp. (°C)	Procedure	Activity ($U\ g^{-1}$)	Temp. (°C)	Procedure	Activity ($U\ g^{-1}$)
DUOLITE ES 568	20	Cov., W.	85	40	Cov., W.	80	40	Cov., H.	48
PVC	20	Cov., H.	85	40	Cov., H.	56	40	Cov., H.	25
Glass AP-685	20	Cov., W.	42	40	Cov., H.	32	40	Ads., Cov., H.	13
Glass Ald-610	20	Ads., Cov., W.	40	40	Ads., Cov., W	10	40	Ads., Cov., H.	6
AP-Al_2O_3	40	Cov., W.	25	40	Cov., H.	35	40	Cov., H.	30

Condition of immobilization: pH 8.0, immob. time 20 h. For methods see the text

with the lipase enzyme immobilized on DUOLITE ES 568 carrier. Further investigations were made with the three immobilized enzymes of maximum lipase activity.

2.6. The stability of immobilized lipases in the operating reactor

The stability of lipase products of maximum activity selected from the trial series was examined for 72 h in a column reactor. The lipase products from *Rhizopus* sp., porcine pancreas and *Penicillium roqueforti* fixed on carrier DUOLITE ES 568 were filled into a column of 10 mm diameter in quantities of 5–5 g, and trioleine substrate, solved in 90% hexane, was circulated through it. Oil content was 10%, pH 8.0, temperature 40 °C and the flowing rate was 10 $cm^3\ h^{-1}$.

Enzyme activity measurement was carried out similarly to the procedure mentioned before. Results are summarized in Fig. 11.

The activity decrease of immobilized enzymes did not surpass 10% continuous operation time of 72 h. Half-life calculated from the initial section of the curve were the followings:

- half-life of immobilized lipase from *Rhizopus* sp.: 21 days, 6 h.
- half-life of immob. lipase from pancreas: 16 days, 1 h.
- half-life of immob. lipase from *Penicillium roqueforti*: 12 days, 4 h.

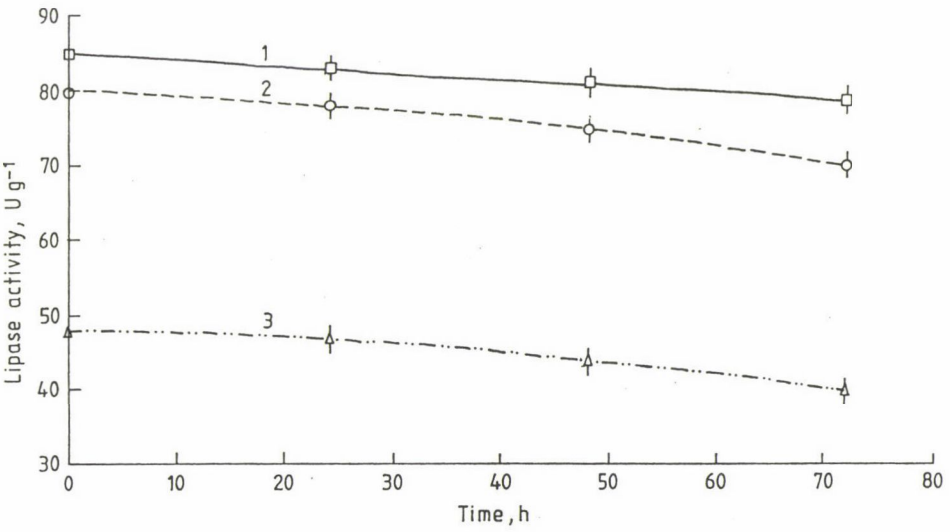

Fig. 11. Activity changes of immobilized lipase preparation on DUOLITE ES 568 support. Immobilized lipase column reactor temperature was 40 °C, substrate solution 10% (v/v) triolein in phosphate buffer and *n*-hexane, at pH 8.0, flow-rate 10 cm^3h^{-1}. 1: R; 2: P; 3: S

3. Conclusions

The use of lipolytic enzymes in food industrial process requires the solution of a series of problems such as enzyme production, immobilization and stability of enzymes originating from different research laboratories. In the trials, we found important to investigate the alternatives of enzyme immobilization as a function of the conditions.

The optimal conditions of immobilization at a temperature higher than the commercial one (40 °C) were determined in relation to pH, carrier and solvent phase.

In the comparison of the enzyme products, the efficiency of integration was not significantly different. There are great differences between the carriers in the lipase activity calculated as per 1 g dry carrier. However, it has to be emphasized that on porous glass carriers, the enzyme can be well immobilized, both with adsorption an with covalent bindings. The study provided base for the realization of a continuous flow method in industrial production.

Abbreviations

Origin of lipase enzyme:	R	*Rhizopus* sp.
	P	porcine pancreas
	S	*Penicillium roqueforti*, culture of the KÉKI Laboratory,
	Cov.	covalent binding
	Ads.	adsorption binding
	W.	water solution
	H.	*n*-hexane solution
	PVC	polyvinil chloride

*

We thank the Ministry of Agriculture for financial help and the Central Research Institute for Chemistry of the Hungarian Academy of Sciences for providing the carrier materials.

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Short communications

SIMULTANEOUS HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF NITRITE AND FORMALDEHYDE FROM FOODS

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A high-performance liquid chromatographic (HPLC) procedure with fluorometric detector has been developed for the determination of nitrite and formaldehyde from foods by use of hydralazine. Hydralazine reacts with nitrit and formaldehyde under acidic conditions in boiling water-bath for 15 minutes to form tetrazolo (5,1-a) phthalazine (Tetra-P) and triazolo (3,4-a) phthalazine (Tri-P) quantitatively. Without extraction, the determination of Tetra-P and Tri-P was simple, specific, sensitive and reliable over the range of 0.003–0.3 mg/kg sodium nitrite and 0.02–0.4 mg/kg formaldehyde, respectively. This procedure using hydralazine is one of the most useful methods for routine analysis of nitrite and formaldehyde in foods, biological fluids and ambient waters.

Keywords: nitrite, formaldehyde, determination, HPLC, foods

The hydralazine (1-hydrazinophthalazine, HP) is an effective depressor of hypertension, reacts with acidified nitrite and formaldehyde to give tetrazolo (5,1-a) phthalazine (Tetra-P) and triazolo (3,4-a) phthalazine (Tri-P) (Fig. 1).

These transformations could be applied to the development of a simple and sensitive method for the determination of nitrite and formaldehyde in foods by means of high-performance liquid chromatography.

For nitrite analysis colorimetric methods using sulphanilic acid (USHER et al., 1975), or sulphanilamide (PHARM. SOC. OF JAPAN, 1980) have generally been used. Gas-chromatographic (GC) methods using *o*-phenylenediamine (AKIBA et al., 1973), or aromatic primary amines for the SANDMAYER reaction and HPLC of nitrite itself without any derivatization (THAYER et al., 1980), were reported. The colorimetric methods lack specificity which is crucially important for analysis because the sample is often turbid and slightly colored. The GC methods generally require complex pre-treatments, and the methods using HPLC for nitrite itself have unsatisfactory detection

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limits. On the other hand, for the analysis of formaldehyde colorimetric determination has generally been used by measuring the condensation products with acetylacetone-ammonium acetate (PHARM. SOC. OF JAPAN, 1980), 4-amino-3-hydrazino-5-mercapto--1,2,4-triazole (AVIGAD, 1983), or chromotropic acid (CHAFETZ et al., 1984). The colorimetry is not necessarily reliable because the samples are sometimes opaque and colored. A GC method using 2,4-dinitrophenyl-hydrazine (SPREITZER & JAGER, 1990), or cyanide ion (IMPROTA et al., 1984), and the HPLC of the condensates with acetylacetone-ammonium acetate (OKAMOTO & YAMADA, 1981) or with 2,4-dinitro--phenylhydrazine (OLSON & SWARIN, 1985) have also been reported. But these require rather complex pre-treatments such as the extraction of a derivative, drying and concentration of the solution prior to the analysis.

In this paper we describe the use of the HPLC method for determination of sodium nitrite and formaldehyde. The method was published by NODA and co-workers (1980, 1986). The preparation of food samples was developed by us.

1. Materials and methods

1.1 Chemicals

Reagent grade HP–HCl was purchased from Sigma (St. Louis, USA), reagent grade sodium nitrite p.a. and reagent grade formaldehyde 35% p.a. from Reanal (Budapest, Hungary). Acetonitrile for the mobile phase was of HPLC grade from Carlo Erba (Milano, Italy). Other chemicals used were of analytical grade.

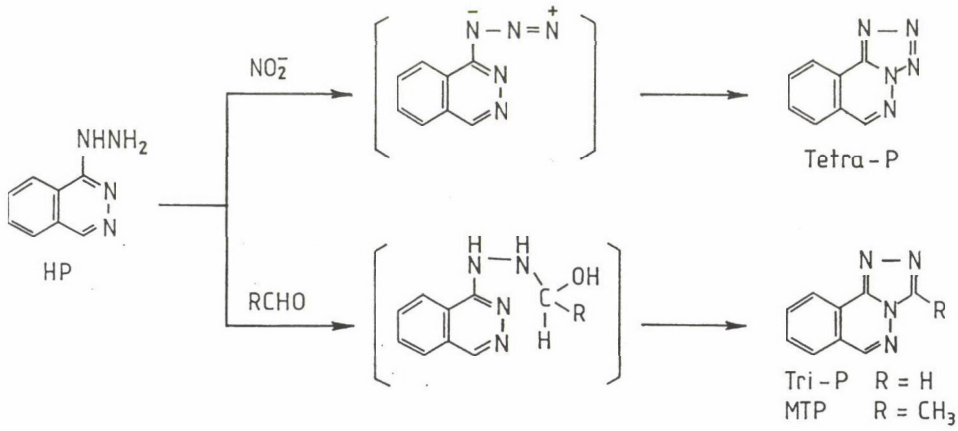

Fig. 1. The reaction schema of nitrite and/or formaldehyde with hydralazine

1.2. Apparatus and HPLC conditions

The prepared food samples were injected through a 20 μl loop (Rheodyne, USA) for analysis on a reversed-phase silica column (Nucleosil-7-C18 2×250×3.9 mm I.D.) (Macherey–Nagel, Düren, Germany) at ambient temperature. The mobile phase (pH cca 4.5) was 30% acetonitrile in 0.05 mol l^{-1} KH_2PO_4 pumped by a Waters Model 501 HPLC pump at a flow-rate of 1.0 ml min^{-1}. Detection was carried out using a Waters 470 type spectrofluorometric detector at 237 nm (excitation) and above 370 nm (emission) wavelengths. The strengthening of detector was 100×.

1.3. Procedures

1.3.1. Standard solution. To prepare standard solutions, sodium nitrite and formaldehyde were dissolved in distilled water. For the recovery study, meat samples were spiked with various amount of sodium nitrite and formaldehyde in the ranges of 3.0–300.0 mg/kg and 20.0–400.0 mg/kg, respectively.

1.3.2. Sample preparation. A 100 ml solution of the food extracts was prepared from 5.0 g of food samples according to the procedure described elsewhere (CSIBA et al., 1995a, 1995b). Accordingly, the matrix materials were removed by 1.0–1.0 ml of Carrez I and Carrez II solutions, respectively.

1.3.3. Determination of sodium nitrite and formaldehyde. A 4.0 ml aliquot of HP hydrochloride (HP–HCl) solution (40 mg in 200 ml of 0.2 mol l^{-1} HCl) was added to 4.0 ml of food extract. The mixture was heated in a boiling water-bath for 15 min. To the cooled reaction mixture 4.0 ml acetonitrile was added. The mixture was passed through filter-paper, if necessary, and 20 μl aliquots were injected directly into the HPLC apparatus.

1.3.4. Calculations. The concentrations of sodium nitrite and formaldehyde in the sample solutions were determined by interpolation from calibration curves, which were constructed by plotting the peak-areas of Tetra-P and Tri-P versus the concentrations of sodium nitrite and formaldehyde (0.003–0.3 ppm and 0.02–0.4 ppm, respectively).

2. Results and discussion

Our test system was sodium nitrite and formaldehyde in 2.5 μg l^{-1} concentrations in distilled water. The blind system was distilled water by itself. To these the hydralazine hydrochlorid was added as described above.

The blind and the spiked medicinal plants chromatograms are presented in Figs. 2 and 3. In Fig. 3, the first and second large peaks are Tri-P (Rt = 12.9 min) and Tetra-P (Rt = 15.6 min) from formaldehyde and sodium nitrite, respectively. The two small peaks before the Tri-P are unknown.

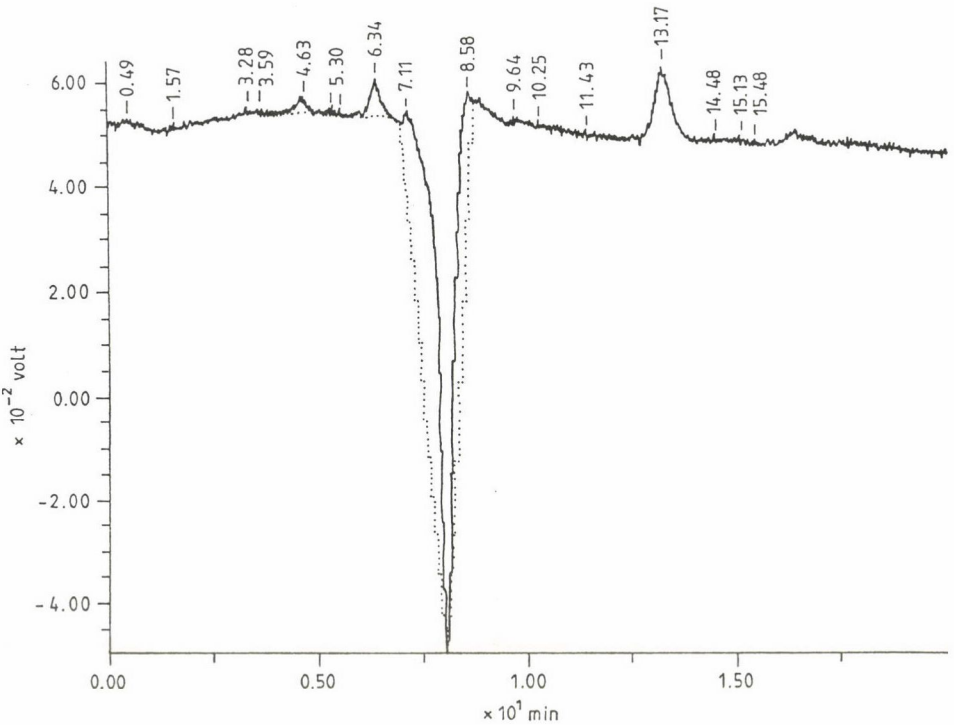

Fig. 2. HPLC chromatogram of the reaction mixture of HP–HCl in water. HPLC conditions: loop volume: 20 μl; column: Nucleosil C18, 2×250×3.9 mm I.D.; mobile phase: 30% acetonitrile in 0.05 mol l^{-1} KH_2PO_4; temperature: ambient; flow-rate: 1.0 ml min^{-1}; detection: fluoroscence: excitation: 237 nm; emission: 370 nm; strengthening: 100 ×

2.1. Analytical data

2.1.1. Linearity. In the examined concentration range (0.15–15.0 ng ml^{-1} for sodium nitrite an 1.0–20.0 ng ml^{-1} for formaldehyde), the detector response was found to be linear for both peak area and peak height measurements.

Calibration equations for sodium nitrite determination are:

$Y = 1.72\ X$, where Y = detector response in V. s,
X = sodium nitrite concentration in mg/l^{-1} sample.

and/or

$Y = 0.086\ X$, where Y = detector response in V.s,
X = sodium nitrite concentration in mg/kg sample

Calibration equations for formaldehyde determination are:

$Y = 1.56\ X$, and/or $Y = 0.078\ X$,
where Y and X symbols are as above.

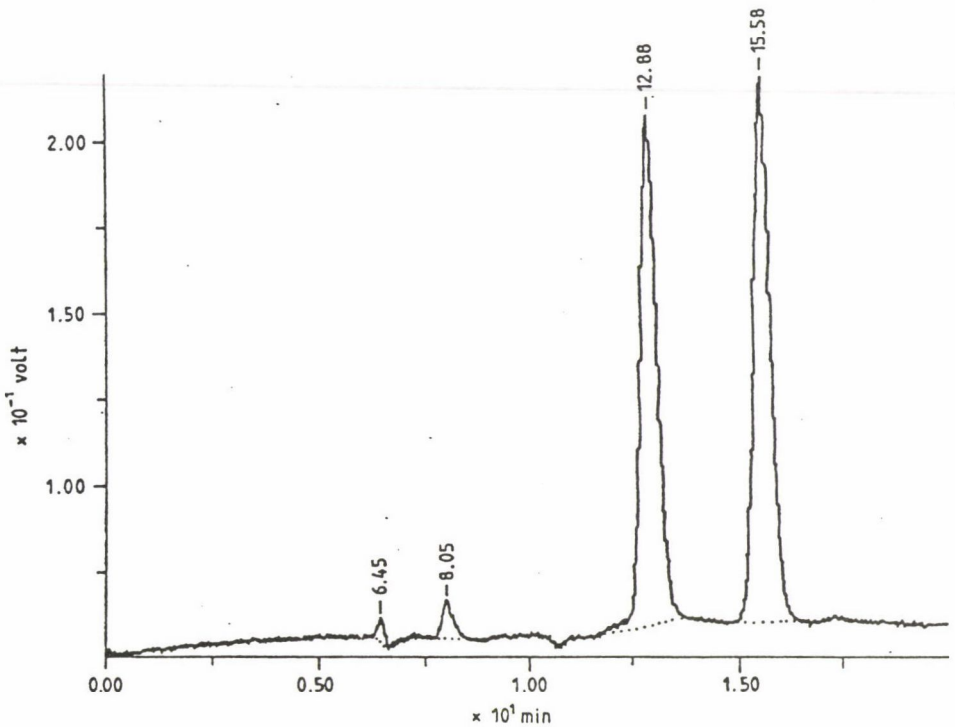

Fig. 3. HPLC chromatogram showing medical plant spiked with 50 ng/g sodium nitrite and formaldehyde before the reaction. Peaks: 1, 2 = unknown; 3 = Tri-P; 4 = Tetra-P HPLC conditions, as in Fig. 2

2.1.2. Limit of detection. The least possible detectable concentration were found to be 0.1 ng/g for sodium nitrite and 0.5 ng/g for formaldehyde, calculated as three times the standard deviation of the noise.

2.1.3. Limit of quantitation. With the sample preparation method the limit of quantification of sodium nitrite and formaldehyde were 3.0 ng/g and 5.0 ng/g, respectively.

2.1.4. Recovery. The recovery of sodium nitrite and formaldehyde was found to be 95±2% in the 20.0–200.0 ng/g concentration range for both compounds.

2.1.5. Precision. The intra-day reproducibility was also studied. The results showed good agreement, with a relative standard deviation of only 0.20% at 10.0 $\mu g\ l^{-1}$ sodium nitrite and/or formaldehyde concentration.

2.2. *Practical application*

Figure 4 shows the HPLC chromatogram of cold plate sample Milanoi. The peak of sodium nitrite is three-times larger than the formaldehyde peak area.

The measured sodium nitrite and formaldehyde concentrations in medicinal plants, milk, cold plates, sausages and canned products are given in Table 1.

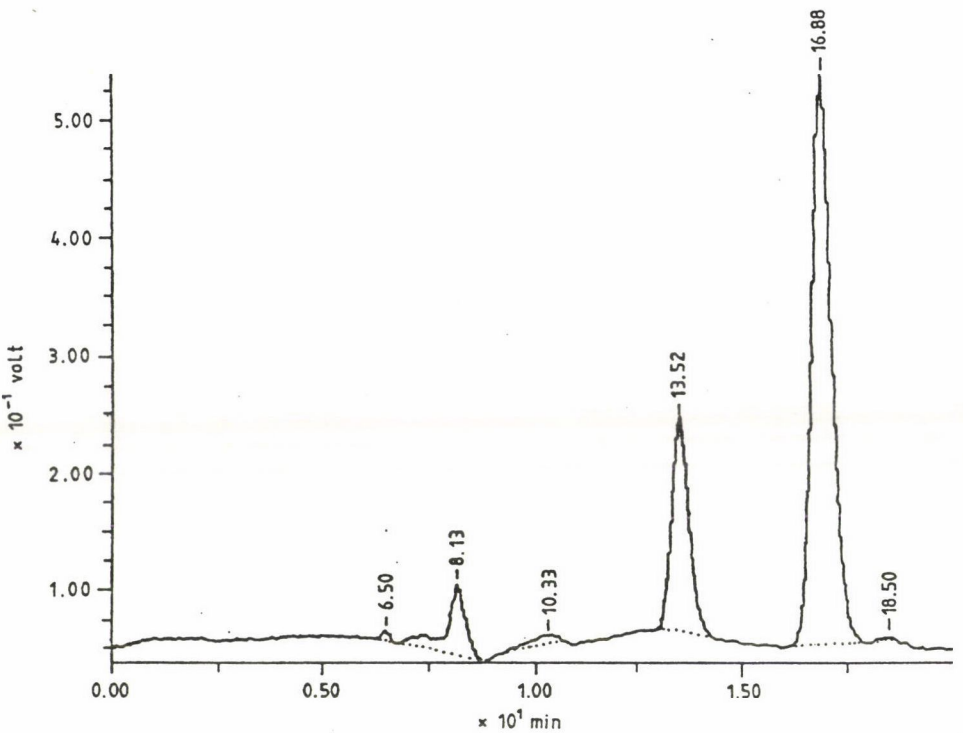

Fig. 4. HPLC chromatogram of sample from cold plate Milanoi. Peaks: 1, 2, 3, 6 = unknown; 4 = Tri-P; 5 = Tetra-P HPLC conditions, as in Fig. 2

The investigated medicinal plants have small nitrite concentration originating from the soil. In case of the cold plates and sausages the source of nitrite is the pickle.

The formaldehyde is always present in biological systems, as endogenous formaldehyde in C1 pool. Exogenous formaldehyde is found in the environmental samples (drinking water, clothes, drug preparation, containers, etc.). The hydralazyne formaldehyde method is not specific. The hydralazyne reacts with other aldehydes and reduced sugars.

Table 1

Analytical results obtained by the proposed method

Sample	Sodium nitrite (mg/kg)	Formaldehyde (mg/kg)
Medicinal plants (Herbaria factory, Hungary)		
Yarrow (Achillea millefolium)	n.d.	4.6
Common comfrey (Symphytum officinale)	n.d.	11.9
Althea root (Altheae officinalis)	4.2	7.2
Cockspur (Crataegus cruss-galli)	n.d.	3.6
Misletoe (Viscum album)	1.2	6.8
Raw milk	1.0	0.5
Cold plates		
Milanoi	29.5	10.0
Párizsi	57.1	10.0
Rakott	78.7	11.1
Sausages		
Casino paprikás	11.6	3.1
Casino	11.6	2.5
Cserkész	11.1	2.9
Canned products		
Liver paste	3.4	1.7
Fish	0.4	3.2
Chopped pork	2.6	0.8
Fish in tomato sauce	0.5	6.2

n.d. = not detectable

An alternative procedure based on the same reaction but using GC was described by TANAKA and co-workers (1981). Their method gave very satisfactory results, that is, very high recoveries of nitrite from foods, milk and blood, and excellent detection limits (0.02 mg/kg, GC–FID; 0.3–4.0 mg/kg GC–ECD). The sensitivity of the GC–ECD method is comparable to that of NODA method, adapted by us. However, the GC method requires more complex pre-treatments (extraction, drying, evaporation, etc.) which are unnecessary in the adapted HPLC procedure. The most striking

characteristics of the HPLC method are the high sensitivity and the simple procedure. In conclusions, the described method is considered to be one of the most useful methods for routine analysis of nitrite and formaldehyde in food and environmental samples.

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A TWO STAGE MODEL DESCRIBES RADIATION SOFTENING OF CARROT*

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Irradiation of food continues to be a subject of considerable scientific interest even though its commercial use is still small. A limiting factor in irradiation of horticultural crops is the softening it causes in fruit or vegetable tissue. Doses above about 1 kGy (100 krad) render the product unacceptably soft (MAXIE et al., 1971).

Vegetable tissue is also softened greatly during thermal processing. HUANG and BOURNE (1983) and BOURNE (1989) showed that thermal softening of vegetable occurs in two stages: a rapid first-order process, followed by a second slow first-order process. To better understand the softening problem we studied the kinetics of radiation softening of raw carrot tissue using modern texture measuring equipment and compared it with the kinetics of thermal softening.

1. Irradiation

Carrots of the 'PY60' cultivar were diced into 9.4 mm cubes and the small pieces removed by sieving. The diced carrot was placed in 250 cm^3 glass beakers which were covered with Saran wrap. The beakers were inserted between an array of twelve 19 cm-long Co^{60} pencils with a total power of 59.2 TBq (1,600 curies), at the Ward Laboratory of Nuclear Engineering at Cornell University. The mass average rate of radiation was 1.73 kGy (173 krad) per h. A series of doses ranging from 0 to approximately 50 kGy (5 Mrad) was given using three beakers of carrot for each dose level. The dose was controlled by the time of exposure to the gamma rays. The firmness was measured in small back extrusion cell mounted in a Lloyd Universal Testing Machine (BOURNE & MOYER, 1968).

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2. Cooking

About 20 kg of prepared dice was dumped into a large steam jacketed kettle of boiling tap water and held at boiling point. At regular intervals an aliquot of approximately 700 g was withdrawn from the kettle and quenched by immersion in cold water. Cooking times ranged from zero up to 300 min.

3. Results

Figure 1 shows the softening curve (shown by the squares) for irradiated carrot by plotting log (extrusion force) versus dose. There is a rapid loss of firmness from 0 to 15 kGy, and then a much slower rate of change from 15 to 52 kGy. Extrapolating the straight line portion of the curve beyond 15 kGy back to zero dose and subtracting this line from the empirical curve above it yields a second straight line with a steep slope (shown by the circles). This shows that radiation softening is consistent with two first-order rate processes.

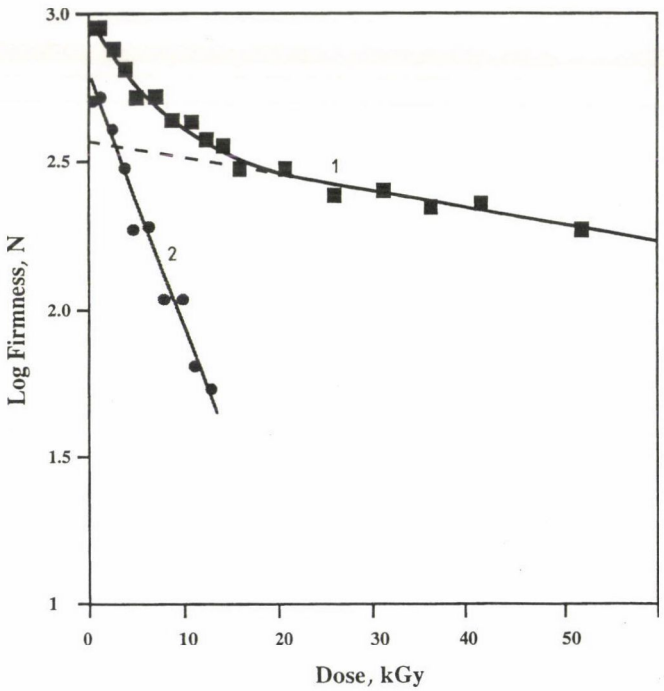

Fig. 1. Effect of radiation on softening of carrot. 1: experimental data points; 2: derived data points

1. fast process: $\ln A = \ln A_0 - K_a t$
2. slow process: $\ln B = \ln B_0 - K_b t$

where A is the amount of the rapidly degraded firmness and B is the amount of the slowly degraded firmness at time t, A_0 and B_0 the amount of the two kinds of firmness in the untreated carrot, K_a and K_b are the apparent first-order rate constants for the two kinds of firmness.

Figure 2 shows the softening curve (shown by the squares) for carrot boiled in water. This also shows a two-stage softening process similar to that first described by HUANG and BOURNE (1983).

1. fast process: $\ln C = \ln C_0 - K_c t$
2. slow process: $\ln D = \ln D_0 - K_d t$

where C is the amount of rapidly degraded firmness and D the amount of slowly degraded firmness at time t, C_0 and D_0 the amount of the two kinds of firmness of the raw carrot. K_c and K_d are the apparent first-order rate constants for the two kinds of firmness.

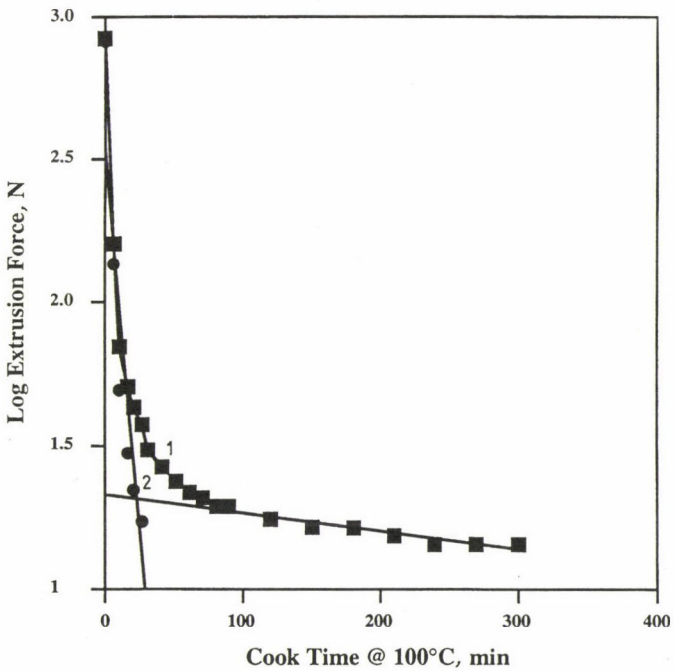

Fig. 2. Effect of cook time on softening of carrot. 1: experimental data points; 2: derived data points

4. Discussion

A comparison of Fig. 1 and Fig. 2 shows that radiation softening and thermal softening of carrot tissue are qualitatively similar because both show a rapid rate of softening at first followed by a slow rate of softening. Therefore, we postulate a simultaneous two substrate, two first-order softening process for radiation softening of vegetable tissue similar to that postulated by HUANG and BOURNE (1983) for thermal softening.

Table 1 lists the apparent first-order rate constants (calculated from the lines of best fit), total firmness, the firmness that is resistant to degradation (intercept on the Y axis), and their ratios. The correlation coefficients (r) for the lines of best fit are shown in parentheses. The high values for r support the postulate of two first-order rate softening processes for both radiation softening and thermal softening.

The intercept of the extrapolated slow process line on the firmness axis gives the amount of the slow softening substrate present. For radiation softening this is 352 N which is 42% of the total firmness while for thermal softening it is 20.9 N which is only 2.5% of the total firmness. For vegetables, this intercept has been called "thermal firmness" because it quantifies the amount of firmness that is resistant to heat degradation (BOURNE, 1987). By analogy we proposed to call the intercept on the radiation-softening curve "radiation firmness" because it quantifies the amount of firmness that is resistant to softening by irradiation (BOURNE, 1995).

Low temperature blanching treatments before thermal processing of vegetables have been shown to increase the amount of thermal firmness, and hence the firmness of canned vegetables (BOURNE, 1989). Future radiation research might profitably explore the possibility of treatments to vegetables before irradiation that will increase the radiation firmness level thereby making vegetables more resistant to radiation softening and removing one of the obstacles to acceptance of irradiated vegetables.

Table 1

Apparent rate constants and amount of firmness resistant to degradation

	Radiation	Cooking
Fast process rate constant	K_a = −0.754 (r = −0.987)	K_c = −0.0563 (r = −0.934)
Slow process rate constant	K_b = −0.00593 (r = −0.968)	K_d = −0.00065 (r = −0.962)
Ratio: Slow rate/fast rate	0.00786	0.0115
Untreated firmness (raw)	848 N	826 N
Y axis intercept	352 N	20.9 N
Firmness ratio: Slow substrate/total	0.415	0.025

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EFFECT OF ELECTRON IRRADIATION ON HATCHABILITY AND BROILER PERFORMANCE OF HATCHING EGGS*

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The irradiation of foods employs the electromagnetic ionizing energy, and the gamma ray, the X ray and the electrons are used. The electrons are reduced mass particles and have negative electric charge. The difference between the gamma rays, X-ray and electron is the penetration level (RAMLER, 1983; DIEHL, 1990). The effective range on penetration of electron accelerator depends on the energy level, in practice the penetration of an electron beam in the foods is 5 mm for MeV (Mega electron Volt) (DIEHL, 1990).

The bacterial contamination of hatching eggs increases the embrionary mortality during the first weeks of life of the chicken and it results in a delay in the growth (PADRON, 1992). Microorganism of extragenital origin is the principal cause of rotting of eggs (BOARD and HALLS, 1973). A relationship between contamination from the shell and hatchability was demonstrated (QUARLES et al., 1970), so the disinfection system is very important to eliminate pathogenic agents. The irradiation effect of hatching eggs on the hatchability and the chicken growth development have intrigued scientists since the beginnings (GERRITS, 1994). It has been known for many years that bacteria, including *Salmonella* can penetrate egg shells (COX et al., 1990). Studies have demonstrated that gamma irradiation of *Salmonella enteritidis* with 1 kGy dose diminished the bacteriological population by 2 log cycles; but the commercial quality of the eggs were altered (MALLET et al., 1993[1]; TELLEZ et al., 1994). ZAKARIA (1989) reported that chicken gotten from egg subjected to low dose of gamma radiation are not affected in their weight. The objectives of this work were: a) to evaluate the effects of electron irradiation on the hatchability and b) to evaluate the productive parameters of chicken hatching from egg irradiated with electrons.

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1. Materials and methods

1.1. Hatching eggs

Eggs were obtained from a 32 weeks old Avian broiler breeder flock.

1.2. Dosimetry

In order to determine the absorbed dose of electron irradiation dosimeter film was used (CHADWICK & ODSTERHEERT, 1986). The radiocromic dye film (MCLAUGHLIN et al., 1988) is commercially known as FWT-60. The dosimeters were cut (1 cm^2) and placed on the outer and inner part of the clean and dry egg shell. The samples of egg shell with the dosimeters were placed on 10 cm of titanium's window in an area of 18 cm. The absorbance values were read before and after irradiation and were compared with a calibration curve for obtaining the absorbed dose in the egg shell. Eggs of the treatment group were irradiated with an electron accelerator type "Pelletron"[2] at a dose rate of about 1.77 kGy min^{-1} and at a window's accelerator distance to egg of 10 cm in an area of 18 cm.

1.3. Experimental design

The eggs were divided in 3 groups, 20 eggs in each for the treatment. The experimental groups were: group 1: irradiated with 1 kGy dose, group 2: irradiated with 2 kGy dose, group 3: non-irradiated control group. Three trials were made for each group.

1.4. Incubation and rearing

After irradiation the eggs were artificially incubated in a commercial hatchery with standard conditions. One day old chicken obtained were individually weighed and reared for 7 weeks, under commercial standard conditions. During raising period the feed was weighed too. The parameters obtained were: average weight, feed conversion, week by consumption of feed and mortality rate.

1.5. Statistical analysis

The data were analyzed by SAS®Software. The results on average weight, feed conversion, feed weekly consumption in the three periods were analyzed by analysis of variance and for the differences between means Duncan test was utilized (LUGINBUKE & SCHLOTZHAVER, 1987).

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The hatchability rate and mortality average were evaluated by Chi-square method.

2. Results

In the experiment there was no significant differences in hatchability and body weight of hatchings among treatments. Feed consumption, gain weight, feed conversion parameters and mortality rate did not demonstrate significant differences among treatments (See Tables 1–6).

Table 1

Hatchability and fertility percentage of eggs irradiated with electrons (two different doses)

Treatment group	Irradiation doses	Born chicken (%)	Fertility (%)
1	1 kGy	90*	98.3
2	2 kGy	90*	93.3
3	Control	91.66	98.3

* There was no significant difference ($P > 0.05$)

Table 2

Causes of embryonic mortality by the embriodiagnosis of eggs irradiated with electrons

Treatment group	Irradiation doses	Defective chicks	Retarded chicks	Early embryonic mortality	Rotting eggs
1	1 kGy	1	0	3	1
2	2 kGy	0	1	1	0
3	Control	1	0	3	0

Table 3

Weight of one day old chicken obtained from electron irradiated eggs (two different doses)

Treatment group	Irradiation doses	Means ± SD (g)	E.E
1	1 kGy	42.034 ± 3.684^a	0.501
2	2 kGy	41.380 ± 2.870^a	0.394
3	Control	42.310 ± 3.164^a	0.422

Means with no common letter differ significantly ($P < 0.05$)

Table 4

Feed consumption during the productive period of chicken obtained from electron irradiated eggs

Treatment group	Irradiation doses	Starter ration means ± SD (g)	Grower ration means ± SD (g)	Finisher ration means ± SD (g)
1	1 kGy	940.59 ± 91.23^a	1218.1 ± 233.47^a	2178.8 ± 93.54^a
2	2 kGy	821.42 ± 28.45^a	1157.9 ± 56.40^a	2226.3 ± 268.7^a
3	Control	842.10 ± 28.46^a	1019.7 ± 83.05^a	2126.6 ± 86.67^a

Means with no common letter differ significantly ($P < 0.05$)

Table 5

Average weight during the rearing chicken from electron irradiated eggs

Treatment group	Irradiation doses	Starter means ± SD	Grower means ± SD	Finisher means ± SD
1	1 kGy	506.0 ± 47.7^a	1055.2 ± 159.1^a	1778.3 ± 253.4^b
2	2 kGy	493.7 ± 50.8^a	1099.9 ± 202.8^a	1889.8 ± 212.1^a
3	Control	497.1 ± 50.0^a	1071.5 ± 117.7^a	1809.1 ± 216.2^{ab}

Means with no common letter differ significantly ($P < 0.05$)

Table 6

Average feed conversion and mortality rate of 7 week old chicken obtained from electron irradiated eggs

Treatment group	Irradiation doses	Feed conversion Means ± SD	Mortality rate (%)
1	1 kGy	2.54 ± 0.28^a	5.55**
2	2 kGy	2.20 ± 0.24^a	1.85**
3	Control	2.22 ± 0.89^a	1.81

Means with no common letter differ significantly ($P < 0.05$)
** There was no significant difference

3. Discussion

The hatchability rate and body weight of hatchings demonstrated that injury is not caused by electron irradiation. Group one and the control group show greater rate of early embryonic mortality due to the inadequate transport. KOSTIN (1960) and KUZIN and co-workers (1963) found an improved hatchability of 2 at 4% at low doses

of gamma irradiation. They irradiated hatching eggs with X-rays (30 to 600 rads) and incubated them for 243 h, and they reported that small dose (<80 rad) accelerated the embryonic development. In other work hatching eggs were irradiated with 50 rad, and an improvement (3.2%) in the hatchability was found. Others reported that low dose (20 rads) of X-rays shortened incubation time (1–2 days) (GERRITS, 1994). Later GERRITS (1994) used 4–17 rads doses of X-rays and observed early hatch of irradiated eggs, although he found more infertile eggs and early embrionary mortality. In this study improved hatchability and early hatch were not observed, in fact, the hatchability diminished by 1.6% in the irradiated groups compared to the control groups. The latest investigation were made with gamma and X ray irradiation with high penetration and when the irradiation doses rose harmful effects were noticed in the embrionary development.

It was reported that 20 rads doses of X-rays shortened the incubation time (1–2 days), about 8% more chicks hatched, 4% lower mortality during rearing and an increased body weight (30 to 60 g) after 6 weeks rearing were found (GERRITS, 1994). These effects were not found in the study and there were no significant differences in the productive parameters after 7 weeks.

The results show that the electron irradiation of hatching eggs is a feasible system for the disinfection of hatching eggs, without any effect on the hatchability and broiler performance.

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GAMMA IRRADIATION OF PEANUT KERNELS TO CONTROL MOLD GROWTH AND TO DIMINISH AFLATOXIN CONTAMINATION

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Growth of *Aspergillus flavus* and *A. parasiticus* and subsequent aflatoxin production represent a serious public health concern to peanut industry and consumers. Since peanuts are a good substrate for aflatoxin-producing molds and the conidia of these fungi are ubiquitous in environments where peanut are grown and stored (ASHWORTH et al., 1965), it is of utmost importance to handle peanut commodities properly in order to protect them from becoming contaminated with aflatoxin. Gamma irradiation, so-called cold sterilization, has been investigated as an alternative process for preserving foods. Elimination of mold contaminations by irradiation affords a means to minimize possibility of aflatoxin formation in foodstuff (BULLERMAN et al., 1973; BULLERMAN & HARTUNG, 1974). The direct effect of gamma irradiation on variations of natural and inoculated mold contaminants, seed germinations, aflatoxin productions, oil properties of peanuts and the subsequent effect on peanut kernels subjected to storage were investigated (CHIOU et al., 1990; 1991).

Populations of the natural mold contaminants present on the uninoculated peanut kernels, before and after irradiation are shown in Table 1. The molds on the kernels were effectively eliminated by a dose greater than 2.5 kGy. At 2.5 kGy, 45% of the kernels still contained viable molds. To test the effectiveness of gamma irradiation on *A. parasiticus* conidia, 20 sound kernels were placed in sterile Petri dishes and then inoculated and evenly mixed with two kernels which had been passed through the surface of a 2-week-old (30 °C) *A. parasiticus* NRRL 2999 culture on potato dextrose agar (PDA). An estimated inoculum of 1.3×10^7 conidia/g peanut was conducted. Then the Petri dishes with inoculated peanuts were irradiated at dose levels of 0, 2.5, 5.0 and 10 kGy before subjected to incubation at a humidified condition. Outgrowth of molds was markedly retarded by irradiation as evidenced by a delay in the appearance

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of visible mycelia on peanut kernel surfaces. The effect was proportionally dependent upon the irradiation dose applied. However, complete destruction of the conidia was not achieved, even at 10 kGy.

Table 1

Growth of natural mold contaminants, seed germination, oil characteristics of uninoculated peanut kernels subjected to gamma irradiation and storage at ambient and frozen temperature for one year and aflatoxin contents of the kernels inoculated with A. parasiticus *and incubated at a humidified condition for 4 weeks*

Items &	Irradiation dose, kGy			
storage	0	2.5	5.0	10
Mold growth on PDA[a] of uninoculated kernels before storage, %				
	100	45.0	0	0
Mold growth on PDA[a] of uninoculated kernels after storage, %				
Ambient	100	2.5	0	0
Frozen	100	0	0	0
Germination of uninoculated kernels before storage, %				
	100	80.9	27.5	6.9
Germination of uninoculated kernels after storage, %				
Ambient	70	0	0	0
Frozen	95	0	0	0
Peroxide content of oils from uninoculated peanuts before storage, OD 500nm				
	0.046	0.085	0.125	0.160
Peroxide value of oils from uninoculated peanuts after storage, OD 500nm				
Ambient	0.132	0.141	0.150	0.167
Frozen	0.084	0.088	0.120	0.148
TBA[b] value of oils from uninoculated peanuts before storage, OD 532nm				
	0.021	0.023	0.037	0.052
TBA[b] value of oils from uninoculated peanuts after storage, OD 532nm				
Ambient	0.030	0.035	0.047	0.062
Frozen	0.022	0.024	0.039	0.046
Aflatoxins of peanut kernels inoculated with *A. parasiticus* and incubated for 4 weeks, μg/g				
B_1	18.63	1.51	12.62	4.28
B_2	2.16	0.09	2.52	0.63
G_1	43.92	0.77	38.36	16.20
G_2	4.41	0.05	3.86	1.17
Total	69.12	2.42	57.36	22.28

[a]PDA: potato dextrose agar; [b]TBA: thiobarbituric acid

After 4 weeks of incubation, aflatoxins B_1, B_2, G_1 and G_2 were extracted, separated by thin layer chromatography and quantitated (Table 1). All peanuts except those irradiated with 2.5 kGy were highly contaminated with aflatoxin. Toxins were non-detected in the uninoculated peanuts. Non-irradiated peanuts inoculated with *A. parasiticus* supported the highest production of aflatoxin. The lower amount of aflatoxin produced on peanuts receiving 2.5 kGy may be attributed to loss of aflatoxigeneity of the inoculum occurring when peanuts were irradiated at the specific dose or growth competition by the surviving natural mold contaminants.

Effects of gamma irradiation on peanut kernels subjected to storage at ambient and frozen conditions

For storage assessment, peanut kernels in polyethylene/nylon bags (100 g/bag) after irradiation with 0, 2.5, 5.0 and 10 kGy were packaged in high-density paper envelopes. The envelopes were stored under ambient (15–31 °C) and frozen (–14 °C) conditions for 1 year. The percentage of peanut kernels contaminated with molds after 1 year storage is shown in Table 1. Regardless of storage temperature, 100% of unirradiated peanut kernels contained molds. When peanut kernels were irradiated with a dose of 2.5 kGy and stored at ambient or frozen conditions, 2.5 and 0% of the kernels, respectively, contained visible molds. Apparently, after irradiation with 2.5 kGy, some of the surviving molds were injured and eventually lost viability during storage. The percentage of peanuts capable of germination was substantially lowered by gamma irradiation (Table 1). About three fourths of the kernels did not germinate after irradiation with 5.0 kGy. After 1 year storage, all gamma irradiated peanut kernels lost germination capabilities. When a comparison was made between irradiated and unirradiated kernels, loss of germination capabilities of the irradiated kernels after storage indicated an immediate effect of gamma irradiation on lowering the germination percentage. In addition, it also resulted in a marked decrease in seed viability during subsequent storage.

After 1 year storage, at each irradiation level, peroxide content in peanuts stored at –14 °C was significantly lower than that in peanuts stored at ambient temperature (Table 1). In peanuts stored at –14 °C, except unirradiated kernels, peroxide contents were close to the original levels subjected to storage. TBA values of peanuts stored at –14 °C were slightly lower than those stored at ambient temperature. TBA values of peanuts stored at –14 °C were about half of those before storage. Electrophoretic patterns of proteins extracted from irradiated and unirradiated peanut kernels after 1 year storage was analyzed by SDS-PAGE. In general, gamma irradiation doses up to 10 kGy did not result in a marked difference in SDS protein patterns. When a

comparison was made between peanuts subjected to a given level of irradiation and stored at ambient temperature and −14 °C, the intensity of the protein bands in peanuts stored at −14 °C was slightly greater than the analogous bands from peanuts stored at ambient temperature.

Conclusion

Peanut kernels inoculated with *Aspergillus parasiticus* conidia and uninoculated kernels were gamma irradiated. Levels higher than 2.5 kGy were effective in retarding the outgrowth of *A. parasiticus* and reducing the population of natural mold contaminants. However, complete elimination of the inoculated mold was not achieved even at the dose of 10 kGy. After 4 weeks incubation of the inoculated kernels in a humidified condition, aflatoxins produced by the surviving *A. parasiticus* were 69.12, 2.42, 57.36 and 22.28 μg/g, corresponding to the original irradiation levels as 0, 2.5, 5.0 and 10 kGy. When uninoculated peanut kernels were irradiated and stored for 1 year at ambient and frozen conditions, molds were detected only on peanuts irradiated with 2.5 kGy and stored at ambient temperature. Peroxide content of peanut oils prepared from the irradiated peanuts increased with increased irradiation dosage. After storage, at each irradiation level, peroxide content in peanuts stored at −14 °C was lower than that in peanuts stored at ambient temperature. TBA values of the oils increased with increased irradiation dosage and changed slightly after storage. The SDS-PAGE protein patterns of peanuts revealed no noticeable variation of protein subunits resulting from irradiation and storage.

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ESTIMATION OF RADIOLYTIC GASES AS A RAPID SCREENING TECHNIQUE TO CONTROL IRRADIATED FOOD*

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(Received: 24 January 1996; accepted: 6 March 1996)

Irradiation of food causes the formation of volatile substances, among them low-molecular gases such as hydrogen, methane, ammonia, carbon monoxide, hydrogen sulfide, carbon dioxide, acetone, dimethylsulfide, etc. These gases are derived from the various food components, such as carbohydrates, fats and proteins, as was reported by PRATT and KNEELAND in 1972. Almost twenty years later, Japanese authors described the estimation of these low-molecular gases, i.e. hydrogen and carbon monoxide, by gas chromatography (GC) for detection of the irradiation treatment of various foods (DOHMARU et al., 1989; FURUTA et al., 1992). Their work was followed up by ROBERTS and co-workers (1992, 1994), HITCHCOCK (1993, 1994, 1995) and DELINCÉE (1993, 1994, 1995, 1996), whereby the latter two authors employed specific gas sensors instead of GC. In our work electrochemical gas sensors are used which usually are employed to monitor gas concentrations in ambient air at working places.

1. Material and methods

Frozen chicken legs and breast chops were purchased in local shops. Part of the breast chops were homogenized to obtain minced meat samples. Irradiation was performed with 10 MeV electrons (Circe III linear accelerator, CGR, MeV Industrie, France). Storage temperature was usually -18 °C.

The chicken meat parts were coarsely chopped (about 50 g), transferred to a stable 1 l glass flask, and the same weight of water was added to ensure even heating. The flask was closed air-tight with a screw cap through which a silicone tubing was

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mounted. The flask was subsequently heated in a microwave oven for about 2 min (visible boiling), and the gases expelled into a gas circuit until 1 l was collected. After collection the gas was pumped around in the circuit using a flow rate of 1 l min^{-1} and the gas stream directed over the specific gas sensors. After a constant measuring value had been achieved (ca. 2.5 min), the sensor values in ppm were recorded, representing the part of the detected gas in the gas stream. Five replicates were analysed for each measuring point. The electrochemical gas sensors (Statotector MWG 2500) were supplied by the "Gesellschaft für Gerätebau" (Dortmund).

2. Results and discussion

In previous work the simultaneous use of multiple gas sensors for carbon monoxide, hydrogen, ammonia and hydrogen sulfide was shown to be promising as a screening test to detect irradiated frozen poultry meat (DELINCÉE, 1995, 1996). The largest changes after irradiation were observed for the carbon monoxide and hydrogen content. Therefore, it was decided in further experiments to measure only these latter gases. Since previously a rather non-specific carbon monoxide sensor with cross-reactivity also to e.g. hydrogen sulfide was employed, now a rather specific sensor filtering out the hydrogen sulfide effects was used.

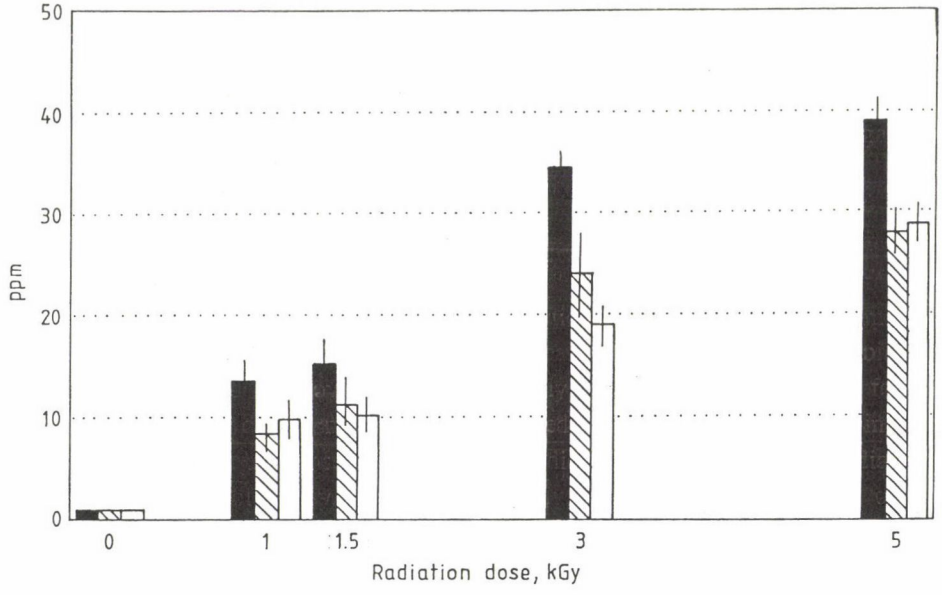

Fig. 1. Hydrogen evolution from irradiated frozen chicken samples, immediately after irradiation (meat values and standard deviations of 5 replicates). ■: Leg; ▨: breast; □: mince

Since structure and chemical composition of the meat may influence gas evolution after irradiation, experiments were carried out on chicken legs, chicken breast chops (containing less fat) and chicken mince (homogenized breast chops) as well.

Hydrogen evolution immediately after irradiation of the frozen chicken samples is shown in Fig. 1. With increasing dose more hydrogen was formed. Similarly, the amount of carbon monoxide increased with dose. However, the quantity of carbon monoxide measured by the newly applied sensor was much less than in previous experiments. The CO levels for 5 kGy samples amounted to about only 5–7 ppm, contrasting to about 30–40 ppm of hydrogen. Frozen storage at –18 °C led to an expected decrease of the trapped low-molecular gases in the samples, probably due to simple diffusion.

Somewhat unexpected, the different chicken samples showed similar decreases during storage, irrespective of their composition or structure (larger surface for the chicken mince). After a period of frozen storage (–18 °C) of 56 days, carbon monoxide values for irradiated samples were equal to those of non-irradiated control samples, whereas the hydrogen content was still slightly higher (just above the detection limit). Since in previous experiments CO levels were higher for irradiated samples than those of non-irradiated ones for several months, some interference may have occurred within the newly applied sensor. More work seems necessary to check interference or cross-reactivity factors of different gas sensors. Possibly the use of a gas sensor microsystem (metal-oxide conductivity detectors made selective by different ceramic coatings or temperature gradients) as described by GOSCHNIK et al. (1993) for the simultaneous determination of a multiple choice of radiolytic gases ("fingerprint") may be of advantage.

3. Conclusion

The rapid estimation of radiolytic gases by sensors is a promising test to detect an irradiation treatment of frozen meat. To ascertain the applicability of this simple and rapid test over the commercially relevant storage periods for the appropriate radiation doses, more research is needed. If elevated gas levels in food samples are measured by the screening test, the indication of the food being radiation processed can be controlled by the more expensive and longer-lasting, but officially validated techniques.

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DNA "COMET ASSAY" FOR RAPID DETECTION OF IRRADIATED FOOD*

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The large molecule of DNA is a particularly sensitive target to ionizing radiations. This is manifested in food irradiation e.g. by the inactivation of microorganisms, killing of insects, inhibition of sprouting and delay in ripening. Since many foods contain DNA, the radiation-induced changes in DNA could serve as a detection method for many food products for evidence of their having been irradiated (DELINCÉE et al., 1993; DELINCÉE, 1996a). A sensitive technique to detect DNA fragmentation is the microgel electrophoresis of single cells, also called "Comet Assay" (MCKELVEY-MARTIN et al., 1993). The application of this technique to detect an irradiation treatment of foods has been described by CERDA and co-workers (1993). One advantage of the Comet Assay is that it is a cheap and simple technique which only lasts a few hours. Previous experiments with the DNA Comet Assay have been carried out with irradiated frozen meats. In an interlaboratory blind trial high rates of identification (91.5%) were achieved (DELINCÉE, 1995a, 1996b). This work describes the application of the DNA Comet Assay to a variety of other irradiated food items e.g. fish, fruits, legumes, seeds and even spices.

1. Experimental

Foods were purchased in local shops. Irradiation was performed with 10 MeV electrons using a Circe III linear accelerator (CGR, MeV Industrie, France). The DNA Comet Assay for animal cells was carried out as previously described (CERDA et al., 1993; DELINCÉE, 1996b). For plant cells slight modifications according to the suggestions of CERDA (1995) were taken into account: plant cells were isolated by grinding in a mortar (fruits, legumes, seeds and spices), a few ml of PBS were added,

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the suspension filtered through a nylon sieve cloth with pore sizes of 250 μm and transferred to small test tubes. After settling for 10–30 min, the supernatant was used as cell suspension, mixed with low-melting agarose (0.8% in PBS) at ca 45 °C at a ratio of 1:10 and spread on pre-coated slides. Lysis was carried out for 15–30 min in 2.5% SDS in a 45 mM Tris-borate, 1 mM EDTA buffer, pH 8. The slides were transferred to conditioning medium consisting of the TBE buffer devoid of SDS. Submarine electrophoresis was performed at 2 V/cm for only 2 min in TBE buffer, pH 8 (without SDS). Silver staining of DNA was carried out as described (DELINCÉE, 1995b).

2. Results and discussion

Although a number of cells with different shapes or lengths of comets could be observed also in non-irradiated samples, intact cells with virtually no or only slight comets were always present in controls. In contrast, irradiated samples showed no intact cells, only cells with comets. These results previously obtained for meat (DELINCÉE, 1996b) could be confirmed for fish (rainbow trout), fruits (strawberries, figs, almonds), legumes (lentils, carioca and macaçar beans), seeds (sunflower, linseed, sesame) and spices (rosé pepper). Frequently just a glance at the slide is enough to decide whether the sample has been irradiated or not. The form of the comets varies with radiation dose, which (with some experience) can be estimated roughly. It should be taken into account, however, that cell nuclei in different plants vary considerably in size and susceptibility against radiation. Sunflower nuclei are quite large, whereas those in sesame are much smaller. Whereas a radiation dose of 0.2 kGy for lentils leads to nice observable comets, similar comets are observed for almonds only at a dose of about 1 kGy. Thus experience has to be gained with individual samples.

In general, however, the qualitative decision whether irradiated or not can be taken just by eye inspection. If necessary, a further evaluation by image analysis can be performed. With some samples problems arose to achieve sufficient lysis of the cell walls, e.g. soya beans or mushroom spores. With shrimps no defined cells or nuclei could hitherto be isolated, probably due to their blanching directly after being caught.

3. Conclusion

The DNA Comet Assay offers potential as an inexpensive and rapid screening test for qualitative detection of irradiation treatment of a wide variety of foods. Suspected samples may subsequently be analysed by more sophisticated, officially validated methods.

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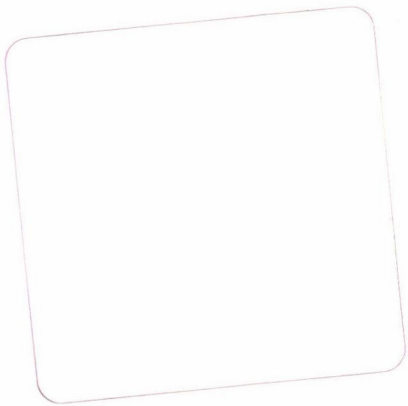

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Evaluation of biochemical and technological quality attributes for 21 durum wheat cultivars grown in Egypt
TAHA, S. A.

Changes of antimicrobial activity in yoghurt during storage
KASALICA, A.

Structural and other alterations on plant tissues caused by quick-freezing and storage time
SÁRAY, T., ZACKEL, E., CSILLAG, A & HORTI, K.

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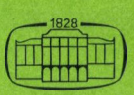

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EVALUATION OF BIOCHEMICAL AND TECHNOLOGICAL QUALITY ATTRIBUTES FOR 21 DURUM WHEAT CULTIVARS GROWN IN EGYPT

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Grain samples of 21 durum wheat cultivars from various world sources, grown under the same conditions in Egypt, were milled into both semolina and whole meal, and subjected to the biochemical and technological tests currently in use. Whole meal samples were analysed for their protein contents, both soluble and insoluble-residue fractions isolated with water, 0.5 mol NaCl, 70% ethanol and 0.1 mol acetic acid were determined. Analysis of variance was carried out to compare the quality attributes of cultivars. Simple correlation coefficients among all quality parameters were calculated to determine the interrelationship of quality parameters. All quality attributes were typically variety-dependent and showed wide ranges of values. Pasta brownness can be predicted by wheat ash and protein contents (specially insoluble), and yellowness by carotene content. Brownness was usually associated with the good cooking quality of pasta. Total protein content and tests relevant to gluten quality (sodium dodecyl sulfate-sedimentation and mixograph characteristics) were strongly associated with each other, but where not efficient to predict pasta cooking quality. Good correlations were found between pasta cooking quality parameters (firmness and tolerance to overcooking) and insoluble-residue protein fractions, specially those isolated with both acetic acid and ethanol, suggesting the possibility of a whole meal index for predicting pasta cooking quality. Such an index is relatively simple, requires only small amounts of grains, and might be used for early generations of durum wheat breeding programs.

Keywords: durum wheat, pasta quality, quality prediction

Durum wheat (*Triticum durum* Desf.) is the cereal of choice for the manufacture of high-quality pasta products. Important attributes of durum wheat, in terms of pasta quality, are milling, colour and cooking quality (SEIBEL & GERSTENKORN, 1993). According to LAIGNELET (1979), good cooking quality and high yellowness are incompatible as brownness can not be separated from good cooking quality. Cooking quality was shown to depend on rheological properties related to gluten strength and on surface characteristics, but these parameters did not seem to be directly related (FEILLET, 1984; AUTRAN et al., 1986; KOVACS et al., 1993, 1995).

Protein quantity and quality of durum wheat are decisive factors of pasta cooking quality. Several reports have linked cooking quality to factors depending on protein content (GALTERIO et al., 1988; D'EGIDIO et al., 1990), gluten quality

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(KOSMOLAK et al., 1980; TAHA & SÁGI, 1987b; D'EGIDIO et al., 1990) and gluten protein solubility fractions (DEXTER & MATSUO, 1978; SGRULLETTA & DE STEFANIS, 1989; STEFANIS et al., 1990).

The emphasis in recent years has been put on breeding wheats for stronger gluten so as to improve rheological properties and cooking quality. A major problem in cultivar development is that no single test has yet existed for predicting cooking quality of early generation material. The mixing characteristics, as determined on a micro-mixograph (BENDELOW, 1967; FINNEY & SHOGREN, 1972) and gluten strength, as determined by sodium dodecyl sulfate (SDS)-sedimentation test (AXFORD et al., 1978), satisfactorily indicate gluten strength, but give poor prediction of cooking quality (DEXTER et al., 1980; D'EGIDIO et al., 1990; KOVACS et al., 1993, 1995). There is, therefore, a pressing need for a standardized simple, fast, small-scale method of pasta quality prediction that can be used for screening high cooking quality durum wheat strains in the early generations in breeding programes. In this context, proteins have been the most widely studied of the grain components, because of their important role in pasta drying and cooking processes (GALTERIO et al., 1988).

This study was undertaken: 1) to compare the quality attributes of 21 durum cultivars grown in Egypt and of different world sources, in order to select out of the cultivars those that show the best quality attributes; 2) to evaluate the relationships between different biochemical and technological variables and pasta quality, in order to define the relative importance of the variables for predicting pasta colour and cooking quality; and 3) to quantitate grain protein fractions isolated with various solvents, in order to identify whole grain parameters associated with cooked pasta quality that might be used in durum wheat breeding programs for quality selection.

1. Materials and methods

1.1. Plant materials

Twenty one cultivars of durum wheat (*Triticum durum* Desf.) employed in this study have been supplied by ICARDA, and adapted under the Egyptian conditions during 1993 and 1994 in the nursery of the Faculty of Agriculture, Zagazig Univ., Egypt. Of the 21 cultivars (harvested in 1994), 12 were from Syria, 6 from Mexico and 3 from Libya. They are listed according to country of origin in Tables 1–4.

1.2. Milling

The grain samples (250 g) were cleaned, conditioned to water content of 14.5%, milled for semolina in a laboratory mill and purified with a laboratory purifier. Crude and pure semolina were weighed and expressed as percentage of whole grain sample.

Whole meals were obtained by dry milling on a Perten laboratory mill 12 V. Particle size were 200–400 μm for semolina and 70–150 μm for whole meal.

1.3. Rheological tests

Gluten strength was measured on whole meal samples from the SDS sedimentation volumes using the method described by AXFORD and co-workers (1978). Mixing characteristics of semolina samples were assessed by the 10-gram micro-mixograph technique of FINNEY & SHOGREN (1972), based upon the parameters: a) mixograph developing time (DT), b) mixograph maximum consistency (MC), and c) mixograph curve area (CA).

1.4. Cooking quality test

Alveograph technique of SCOTTI and co-workers (1976) was used as follows: Ten discs (5 cm diameter) of dried pasta were prepared from pure semolina samples. Then, 5 discs were normally cooked (15 min) and the other 5 discs were over-cooked (30 min). Alveograph values were measured on the discs cooked for 15 min and 30 min to determine the firmness of normally cooked pasta (F15) and tolerance of pasta to overcooking (F30), respectively. The sum of the two values indicates the pasta total firmness (FT). The percentage of firmness loss by overcooking (FL%) was calculated from the equation:

$$FL\% = \frac{F15-F30}{F15} \times 100$$

1.5. Colour score

Semolina yellow index (YI), semolina brown index (BI) and colour class were measured on wet pasta discs prepared from pure semolina according to the adapted method of ALAUSE and FEILLET (1970) as previously described by TAHA and SÁGI (1987a). A well coloured pasta is characterized by low brown and high yellow indexes.

1.6. Protein fractionation

Protein solubility fractions were isolated with four different solvents as follows:

1.6.1. Water. A 0.5 g sample of whole meal was suspended in 5 ml of distilled water and stirred for 2 h. After stirring it was centrifuged for 20 min at 4000 r.p.m. The residue was resuspended in 3 ml of water and stirred again for 2 h, followed by centrifugation for 20 min at 4000 r.p.m. The supernatants, containing water-soluble (W-S) proteins were combined, and analysed for their protein content by the biuret

method. The water-insoluble residue (W-IR) was then stored at -20 °C until the determination of its protein (maximum 20 days).

1.6.2. NaCl. A 0.5 g sample of whole meal was suspended in 5 ml of 0.5 mol NaCl solution and stirred for 2 h, and then centrifuged for 20 min at 4000 r.p.m. The supernatant was collected. The pellets were extracted once more as before and the supernatants were combined. This procedure gave salt-soluble (S-S) proteins (albumins+globulins) and salt-insoluble residue (S-IR) protein fractions.

1.6.3. Ethanol. A 0.5 g of sample was extracted with 70% aqueous ethanol according to a procedure similar to that described in water extraction. This method gave ethanol-soluble (E-S) and ethanol-insoluble residue (E-IR) protein fractions.

1.6.4. Acetic acid. The acetic acid-soluble (AA-S) protein fraction was extracted twice with 0.1 mol acetic acid by the same procedure as previously described. The pellets containing the acetic acid-insoluble residue (AA-IR) protein fraction were then stored at -20 °C for 10–20 days.

1.7. Chemical analyses

The protein content of the soluble fractions isolated with water, 0.5 mol NaCl, 70% ethanol and 0.1 mol acetic acid was determined by the biuret method by adding 1.0 ml of protein solution to 4.0 ml of biuret reagent. The absorbance was measured at 540 nm on Spectronic-20 spectrophotometer. The biuret reagent consisted of 3 g of $CuSO_4 \cdot 5\ H_2O$, 9 g of sodium-potassium tartarate, 8 g of sodium hydroxide, and 5 g of potassium iodide, all dissolved in water and made up to 1 l.

The protein content of whole meal samples as well as that of the insoluble residues isolated with various solvents was determined by the micro-Kjeldahl method according to A.A.C.C. method 46-10 (1983). Moisture and total ash content were determined according to the A.A.C.C. methods No. 44-15A and 08–03, respectively (A.A.C.C., 1983). Carotenoid pigments were extracted overnight from 8 g of semolina with 40 ml of water-saturated n-butanol and the light absorbance of the clear filtrates was spectrophotometrically measured at 440 nm according to A.A.C.C. methods (1968).

1.8. Statistical analyses

Raw data were subjected to computerized two-way (randomized complete block design) analysis of variance (ANOVA), followed by Duncan's multiple range test (DUNCAN, 1955). Correlation coefficients between quality parameters were also calculated (SVCB, 1981). The presented data are the averages of at least two parallel determinations.

2. Results and discussion

2.1. Cultivars and their quality attributes

Mean, grand mean, range of variability values of all the chemical and technological quality tests (25 variables) measured on 21 durum wheat cultivars grown in Egypt, and of different world sources are presented in Tables 1–4. Significancy of variances (*F*-test) is also given. The variability values well reflect the qualitative diversity of durum cultivars under investigation. These confirm that most quality tests of durum wheat, such as rheological properties, SDS-sedimentation volume, semolina yellow index (YI) and cooking characteristics are essentially varietal-dependent (LAIGNELET et al., 1972; DEXTER et al., 1981).

Examining the ranges of the specific quality attributes of the 21 studied durum cultivars, the results (Tables 1–4) indicate the broad spread of quality in this set of genotypes, justifying the claim that they represent broad genetic base with respect to dough properties and pasta quality (KAAN, et al., 1993). All quality parameters showed a range of values. We therefore, had a set of samples with a wide range of quality attributes, suitable for comparative studies.

2.1.1. Chemical composition and colour components. The data reported in Table 1 reveal that Lahn and Om Rabi 5 cultivars showed the highest and lowest protein contents (19.9 and 13.2%), respectively. However, Lahn, Haurani, Awl 2/BIT and Ru/MRB 18 can be considered as cultivars having the highest protein content, in spite of the significant differences between them. Ash content is an important factor for predicting pasta colour, particularly brownness. Lahn and Daki showed the highest and lowest ash contents, respectively. Two cultivars, Haurani and Om Rabi 5, had the highest carotenoid pigment content, 12.7 and 12.1 ppm, respectively. However, Haurani showed lower semolina – YI, higher semolina – BI values and bad colour class, while Om Rabi 5 showed higher YI, lower BI and superior colour class. This may be explained through the differences in oxidative enzyme activity levels, such as peroxidase, polyphenoloxidase and lipoxygenase that destroy semolina carotenoid pigments and produce brown phenolic compounds during pasta processing (MATSUO et al., 1970; LAIGNELET et al., 1972; LAIGNELET, 1983; TAHA & SÁGI, 1987a). Also it may be due to the differences in protein or ash contents, since significant relationships were found between colour score and protein or ash contents (KOBREHEL et al., 1974; TAHA & SÁGI, 1987a).

Table 1

Chemical composition (on d. w. basis) and colour score of 21 durum wheat cultivars grown in Egypt

Source and cultivar	Whole wheat						Semolina					
	Moisture (%)		Protein (N×5.75) (%)		Ash (%)		Carotene (ppm)		Colour score			
									Yellow index		Brown index		Class
Syria													
Haurani	9.77	BC	19.1	B	1.41	B	12.7	A	30.5	EF	33.2	A	IV
Brachoua	9.55	DEFG	14.9	FG	1.11	C	10.4	D	30.0	G	33.5	A	IV
Om Rabi 5	9.50	FG	13.2	J	0.92	G	12.1	B	31.9	B	26.8	L	I
Belik H2	9.57	DEFG	15.5	E	1.03	E	8.2	H	27.8	I	28.6	J	III
Gureau 1	9.56	CDEF	15.4	EF	0.97	F	6.4	I	27.4	J	30.2	GH	IV
Cedifla	9.71	CD	13.3	J	0.83	I	9.4	F	30.1	G	26.3	MN	I
Ruff	9.55	DEFG	14.4	GH	0.78	JKL	3.8	K	21.2	N	27.0	L	VI
Cham 1	10.10	A	13.7	IJ	0.76	LM	9.1	FG	31.2	C	26.3	MN	I
Awl 1/MRB20	9.52	EFG	16.3	D	0.84	I	6.8	I	25.9	L	29.8	HI	IV
Awl 2/BIT	9.25	H	17.3	C	0.75	M	10.7	CD	32.4	A	32.7	B	III
Ru/MRB 18	9.65	CDEF	16.8	C	0.75	M	8.9	G	31.2	C	31.2	D	III
Ru/MRB 15	9.70	CD	14.3	H	0.77	JKLM	8.3	H	28.0	I	27.8	K	III
Libya													
Om Rabi 3	9.46	G	15.9	DE	0.79	J	10.5	D	31.1	CD	31.1	DE	III
Om Rabi 9	9.67	CDE	14.1	HI	0.89	H	11.0	C	31.3	C	30.7	EF	II
Tensift 1	9.45	G	14.0	HI	1.09	B	8.0	H	26.9	K	26.0	N	III

Table 1 (cont.)

Source and cultivar	Whole wheat						Semolina								
	Moisture (%)		Protein (N×5.75) (%)		Ash (%)		Carotene (ppm)		Colour score						
									Yellow index		Brown index		Class		
Mexico															
Lahn	9.57	DEFG	19.9	A	1.45	A	9.9	E	30.8	DE	32.1	C	IV		
Chahda 88	9.48	G	14.0	HI	0.70	O	9.4	F	31.7	B	29.5	I	I		
Snip/Fg/Ato	10.19	A	15.6	E	0.78	JK	5.8	J	25.1	M	28.5	J	IV		
Cham 3	9.23	H	14.0	HI	0.76	KLM	9.1	FG	29.5	H	26.6	LM	I		
Daki	9.91	B	14.3	H	0.72	N	9.8	E	30.4	F	30.4	FG	III		
Ofm/Somo	9.06	I	14.1	HI	0.83	I	6.6	I	27.0	K	27.9	K	III		
Range	9.06–10.19		13.2–19.9		0.70–1.45		3.8–12.7		21.2–32.4		26.0–33.5				
Grand mean	9.60		15.22		0.90		8.87		29.09		29.31				
F-test	29.29***		106.44***		462.63***		340.90***		706.42***		312.53***				

Means in the same column followed by the same letter are not significantly different as $P<0.05$ by DUNCAN's (1955) multiple range test.

*** Significant at P=99.9% probability level.

Table 2

Semolina milling yield, gluten strength and mixing properties of 21 durum wheat cultivars grown in Egypt

Source and cultivar	Milling extraction				SDS-sed. volume (ml)		Mixograph					
	Crude semolina (%)[a]		Pure semolina (%)[a]				Developing time (min)		Maximum consistency (Mu)		Curve erea (cm^2)	
Syria												
Haurani	68.9	H	54.0	GH	32	B	59	D	710	A	36.4	A
Brachoua	76.2	BCDEF	59.0	D	28	C	63	C	491	H	26.6	D
Om Rabi 5	77.4	ABCD	61.7	B	18	H	52	FG	500	G	19.8	H
Belik H2	73.3	G	57.7	E	34	A	79	A	500	G	32.0	B
Guerau 1	78.5	ABC	61.3	B	15	K	48	H	412	L	20.0	GH
Cedifla	79.1	A	59.6	CD	22	E	70	B	395	M	25.9	DE
Ruff	77.1	ABCDE	61.5	B	16	J	53	FG	439	K	19.5	H
Cham 1	78.7	ABC	55.0	FG	12	M	41	J	395	M	12.6	KL
Awl 1/MRB20	74.0	FG	53.5	H	21	F	41	J	517	E	16.6	J
Awl 2/BIT	76.0	CDEFG	51.0	I	16	J	40	J	544	C	17.0	IJ
Ru/MRB 18	78.9	AB	51.9	I	16	J	48	H	509	F	21.4	F
Ru/MRB 15	74.6	EFG	51.1	I	16	J	45	I	474	J	17.4	IJ
Libya												
Om Rabi 3	77.0	ABCDE	54.0	GH	18	H	51	G	526	D	21.9	F
Om Rabi 9	76.0	CDEFG	56.1	A	17	I	48	H	526	D	21.5	F
Tensift 1	78.7	ABC	65.5	A	20	G	56	E	412	L	21.0	FG

Means in the same column followed by the same letter are not significantly different as $P<0.05$ by DUNCAN's (1955) multiple range test.

[a]: Percentage of whole grain sample.

***: Significant at P=99.9% probability level.

Table 2 (cont.)

Source and cultivar	Milling extraction		SDS-sed. volume (ml)	Mixograph		
	Crude semolina (%)[a]	Pure semolina (%)[a]		Developing time (min)	Maximum consistency (Mu)	Curve area (cm^2)
Mexico						
Lahn	74.6 EFG	60.5 BC	28 C	54 EF	631 B	30.2 C
Chahda 88	75.5 DEFG	57.5 E	10 N	44 I	351 N	12.9 K
Snip/Fg/Ato	77.2 ABCDE	54.8 FG	22 E	59 D	517 E	25.3 E
Cham 3	78.6 ABC	53.1 H	20 G	51 GH	482 I	20.1 GH
Daki	76.9 ABCDE	55.5 F	14 L	48 H	412 L	11.5 L
Ofm/Somo	74.0 FG	51.0 I	24 D	52 FG	439 K	17.8 I
Range	68.9–79.1	51.0–65.5	10.0–34.0	40.0–79.0	351–710	11.5–36.4
Grand mean	76.22	56.86	19.93	52.45	484.86	21.30
F-test	9.13***	132.78***	3339.60***	96.42***	1692.89***	255.69***

Means in the same column followed by the same letter are not significantly different as $P<0.05$ by DUNCAN's (1955) multiple range test.

[a]: Percentage of whole grain sample.
***: Significant at P=99.9% probability level.

2.1.2. Milling yield and rheological properties. As it is obvious from Table 2, SDS-sedimentation volumes ranged between 10 and 34 ml for Chahda 88 and Belik H2 cultivars, respectively. Belik H2, Haurani, Brachoua and Lahn cultivars, respectively, showed higher SDS-sedimentation volumes. Mean value of these four cultivars (30.5 ml) exceeds 150% over the grand mean value (19.9 ml) of the 21 examined cultivars.

Concerning the rheological properties determined by mixograph technique, the dough of Belik H2 cultivar, which showed the highest SDS-sedimentation volume, also needed the longest time for developing (mixograph-DT). Regarding the mixograph-MC and CA parameters, Haurani cultivar showed the highest values. It can be noticed that this cultivar (Haurani) had also higher levels for SDS-sedimentation volume, protein, ash, carotene and BI, which may predict good pasta cooking quality, but with bad colour score, according to LAIGNELET (1979) and TAHA and SÁGI (1987a).

2.1.3. Cooking quality. The data presented in Table 3 show the cooking quality parameters determined as alveograph value (bar). These results revealed that Awl 2/BIT, Snip/Fg/Ato, Ruff and Brachoua cultivars exhibited the highest firmness values of normally (15 min) cooked pasta. The mean alveograph value for these four cultivars (139) was 23% higher than the grand mean (113). Four cultivars (Awl 2/BIT, Brachoua, Om Rabi 3, Om Rabi 5) had the highest tolerance to overcooking up to 30 min. Cultivars showing lower loss of firmness by overcooking are; Tensift 1, Brachoua, Awl 2/BIT and Om Rabi 5. Accordingly, the latter three cultivars can be recommended as the best ones for overall cooking quality, because they exhibited the highest values for firmness and tolerance to overcooking as well as the lowest values for firmness loss by overcooking.

2.1.4. Protein solubility fractions. The quantitative solubility protein data of the cultivars investigated in this study, fractionated with water, 0.5 mol NaCl, 70% ethanol and 0.1 mol acetic acid are shown in Table 4. These results clearly reflect the quantitative diversity of protein solubility fractions among the examined cultivars. Albumins, globulins, gliadins and soluble glutenins are known as protein fractions soluble in water, salts, alcohols and acids, respectively, and insoluble glutenins are the acid-insoluble residue (BUSHUK, 1985; CHAKRABORTY & KHAN, 1988; KHAN et al., 1989). Accordingly, globulins+gliadins+glutenins, gliadins+glutenins and glutenins are the expected insoluble residues isolated from water, 0.5 mol NaCl and 70% ethanol, respectively. The results presented in Table 4 revealed that among all cultivars, except for Haurani, the 70% ethanol-soluble (E-S) fraction gave the highest protein content followed by 0.1 mol acetic acid soluble (AA-S), 0.5 mol NaCl-soluble (S-S) and water-soluble (W-S) fractions, respectively, and vice versa as regarding the

insoluble residue (IR) fractions. The relative protein contents of the insoluble residue fractions isolated with water, NaCl, ethanol and acetic acid represent about 74, 71, 42 and 53 percent of the total protein, respectively.

Table 3

Cooking quality parameters of 21 durum wheat cultivars grown in Egypt

Source and cultivar	Alveograph value (bar)						Firmness loss by overcooking (%)	
	Cooking time				Total			
	Normal (15 min)		Over (30 min)					
Syria								
Haurani	113	I	71	FG	104	G	37.2	HIJ
Brachoua	131	D	91	B	222	B	31.0	KL
Om Rabi 5	122	G	83	CD	205	DE	32.4	JKL
Belik H2	111	J	55	H	166	HI	50.5	BC
Guerau 1	96	N	37	L	133	L	61.3	A
Cedifla	102	KL	51	HIJ	153	IK	50.0	BC
Ruff	135	C	73	EF	208	CD	46.4	CDE
Cham 1	71	P	45	K	116	M	36.2	HIJK
Awl 1/MRB20	103	K	55	HI	158	IJ	47.1	CD
Awl 2/BIT	151	A	103	A	253	A	31.9	KL
Ru/MRB 18	124	F	70	FG	194	F	43.3	DEFG
Ru/MRB 15	119	H	70	FG	189	FG	41.2	EFGH
Lybia								
Om Rabi 3	124	F	84	C	208	CD	32.0	JKL
Om Rabi 9	99	M	46	JK	145	K	53.3	B
Tensift 1	100	M	73	EF	172	H	27.2	L
Mexico								
Lahn	118	H	71	FG	189	FG	39.1	GHI
Chahda 88	82	O	49	IJK	131	L	39.9	FGHI
Snip/Fg/Ato	139	B	78	DE	216	BC	44.0	DEFG
Cham 3	101	L	66	G	167	H	35.2	IJK
Daki	114	I	68	FG	182	G	40.2	FGHI
Ofm/Somo	126	E	70	FG	196	EF	44.4	DEF
Range	71–151		37–103		116–253		27.2–61.3	
Grand mean	113.14		66.91		180.05		41.11	
F-test	120.41***		64.99***		130.80***		23.00***	

Means in the same column followed by the same letter are not significantly different as $P<0.05$ by DUNCAN's (1955) multiple range test.

***: Significant at P=99.9% probability level.

Table 4

Protein fractions isolated with various solvents for 21 durum wheat cultivars grown in Egypt

Source and cultivar	Protein content (% on dry weight basis)															
	Water				0.5 mol NaCl				70% Ethanol				0.1 mol acetic acid			
	Soluble		Insoluble		Soluble		Insoluble		Soluble		Insoluble		Soluble		Insoluble	
Syria																
Haurani	2.51	B	14.08	AB	5.75	A	13.63	A	10.33	A	8.45	BC	9.15	I	9.50	CD
Brachoua	1.67	HIJ	13.08	BC	3.33	HI	11.73	BC	6.61	H	8.10	CD	5.18	FG	9.69	CD
Om Rabi 5	1.74	GHI	9.59	EF	3.30	I	9.50	FG	7.19	FGH	5.88	GH	5.21	FG	7.82	F
Belik H2	2.05	E	11.61	D	3.93	DEF	11.16	CD	9.25	BC	6.10	G	7.60	C	7.55	FG
Guerau 1	2.13	DE	9.82	EF	3.85	DEFG	10.09	EF	10.63	A	4.58	I	7.92	BC	6.48	IJ
Cedifla	1.76	FGH	10.64	E	3.93	DEF	8.58	H	8.45	D	4.61	I	6.62	DE	6.56	IJ
Ruff	1.60	IJ	12.64	C	3.60	GFHI	10.37	E	7.02	GH	7.13	EF	5.04	G	9.25	D
Cham 1	1.59	J	7.94	G	4.05	D	8.79	H	9.32	BC	4.00	J	7.93	BC	5.40	K
Awl 1/MRB20	2.54	B	10.23	E	4.95	C	10.66	DE	10.40	A	5.57	H	8.78	A	7.25	GH
Awl 2/BIT	2.18	DE	14.79	A	3.66	EFGH	13.86	A	7.62	FG	9.42	A	5.08	G	11.96	A
Ru/MRB 18	2.22	CD	12.75	C	4.80	C	11.10	CD	9.06	C	7.42	E	6.62	DE	9.83	C
Ru/MRB 15	1.89	F	10.56	E	3.58	GHI	10.30	E	8.42	D	5.65	H	6.33	E	7.77	F
Lybia																
Om Rabi 3	1.89	F	13.96	AB	3.98	DE	11.45	C	7.78	EF	7.85	D	6.26	E	9.45	CD
Om Rabi 9	1.65	HIJ	8.29	G	3.50	HI	10.08	EF	8.32	DE	5.50	H	6.88	D	6.90	HI
Tensift 1	1.63	HIJ	8.80	FG	3.48	HI	10.01	EF	8.93	CD	4.77	I	6.49	E	6.16	J

Table 4 (cont.)

Source and cultivar	Protein content (% on dry weight basis)											
	Water		0.5 mol NaCl		70% Ethanol		0.1 mol acetic acid	
	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
Mexico								
Lahn	2.93 A	14.47 A	5.28 B	13.33 A	10.79 A	8.76 B	8.96 A	10.62 B
Chahda 88	2.25 CD	8.22 G	3.50 HI	9.08 GH	9.70 B	4.10 J	8.12 B	5.51 K
Snip/Fg/Ato	2.35 C	13.20 BC	2.88 J	12.21 B	6.82 H	8.45 BC	4.55 H	10.80 B
Cham 3	1.85 FG	10.21 E	3.50 HI	10.08 EF	8.32 DE	5.50 H	7.63 C	6.20 J
Daki	1.89 F	10.56 E	3.58 GHI	10.30 E	8.45 D	5.62 H	6.97 D	7.05 H
Ofm/Somo	1.65 HIJ	12.29 CD	2.63 J	11.08 CD	6.83 H	6.88 F	5.48 F	8.30 E
Range	1.59–2.93	7.94–14.79	2.63–5.75	8.58–13.86	6.61–10.79	4.00–9.42	4.15–8.96	5.4–11.96
Grand mean	1.999	11.32	3.86	10.828	8.583	6.397	6.56	8.098
F-test	52.23***	43.14***	56.43***	48.73***	44.32***	160.02***	145.77***	155.62***

Means in the same column followed by the same letter are not significantly different as $P<0.05$ by DUNCAN's (1955) multiple range test.

***: Significant at P=99.9% probability level.

The cooking quality of pasta made from widely different durum varieties can differ, due to differences in the structure of their gluten proteins. This is generally referred to as protein "quality" for pasta processing. Most of the variations in protein quality appears to result in from intervarietal differences in glutenin proteins as reflected, for example, by differences in solubility in various solvents found for the 21 cultivars of durum wheat investigated in the present study.

Differences in solubility of proteins can result in from a combination of differences in submolecular, molecular, and supermolecular properties, such as: 1) amino acid composition, i.e. the number of chemical groups, such as disulfide bonds and sulfhydryl and amide groups, would be especially important; 2) molecular weight and molecular weight distribution; 3) subunit composition; and 4) aggregative tendency with other proteins and nonprotein constituents. Primary structure or amino acid sequence would be particularly important in this regard. Long sequences of hydrophobic amino acids would lead to strong association through cooperative hydrophobic interactions, and terminal cysteine residues would lead to rheologically highly active disulfide crosslinkages (BUSHUK, 1985).

2.2. Correlations between quality tests

Simple correlation coefficients, calculated between all possible quality parameters for the duplicate analysis of 21 cultivars, are presented in Fig. 1. Four main general observations were made:

1. Although the first and second mixograph parameters (DT and MC) were significantly correlated (r=0.75 and 0.69, respectively) to the third one (CA), they did not associate with each other (r=0.12, ns). The three mixograph parameters, i.e. DT, MC and CA strongly correlated with SDS-sedimentation volume (r=0.88, 0.62 and 0.75, respectively) supporting previous reports which demonstrated that SDS-sedimentation volume gave a good prediction of gluten strength (DEXTER et al., 1980; 1981; MATSUO et al., 1982; AUTRAN et al., 1986; KOVACS et al., 1993, 1995).

2. Parameters related to gluten quality, SDS-sedimentation volume and mixograph characteristics (except MC), did not directly relate to cooking quality parameters (i.e. F15, F30 and FL%) this statement is in good agreement with previous reports (FEILLET, 1984; AUTRAN et al., 1986; KOVACS et al., 1993, 1995). Cooking quality parameters showed significant correlations with mixograph-MC and low correlations with protein content except tolerance to overcooking (F30) which showed no significant

correlation. This confirmed previous investigations (DEXTER & MATSUO, 1980; MATSUO et al., 1982; AUTRAN et al., 1986), which conclusively demonstrated that cooking quality depends on both gluten strength and protein content.

3. Wheat protein and ash contents were significantly associated ($r=0.62$) with each other, as previously reported by MATSUO and co-workers (1982), and both ash and protein contents tended to be strongly correlated with mixograph-CA, mixograph-MC, SDS-sedimentation volume and semolina-BI. Highly significant correlations between semolina BI and both wheat protein and ash contents have also been found by KOBREHEL and co-workers (1974) and TAHA and SÁGI (1987a). The association of protein content and pasta brownness may be explained by the fact that higher protein content is associated with higher oxidative enzyme activities which are responsible for carotene destruction and formation of brown phenolic compounds during pasta processing (KOBREHEL et al., 1974; LAIGNELET, 1979). A very highly significant positive correlation ($r=0.89$) was found between semolina carotene content and YI, which is in total agreement with previous reports (LAIGNELET et al., 1972; TAHA & SÁGI, 1987a), whereas both did not show correlations with any of the other quality parameters, as has also been stated by AUTRAN and co-workers (1986).

4. As expected, protein quantities of all protein fractions isolated with various solvents (except AA-S) strongly correlated with wheat total protein content. SDS-sedimentation volume showed highly significant positive correlations with both W-IR and S-IR, but lower with both E-IR and AA-IR. No correlation was found between mixograph DT and any of the protein solubility fractions. Whereas both mixograph-MC and CA, as well as semolina brownness significantly correlated to all protein fractions, except to both E-S and AA-S fractions, supporting that brownness can not be separated from good cooking quality, as it was previously concluded (LAIGNELET, 1979; MATSUO et al., 1982; TAHA and SÁGI, 1987a).

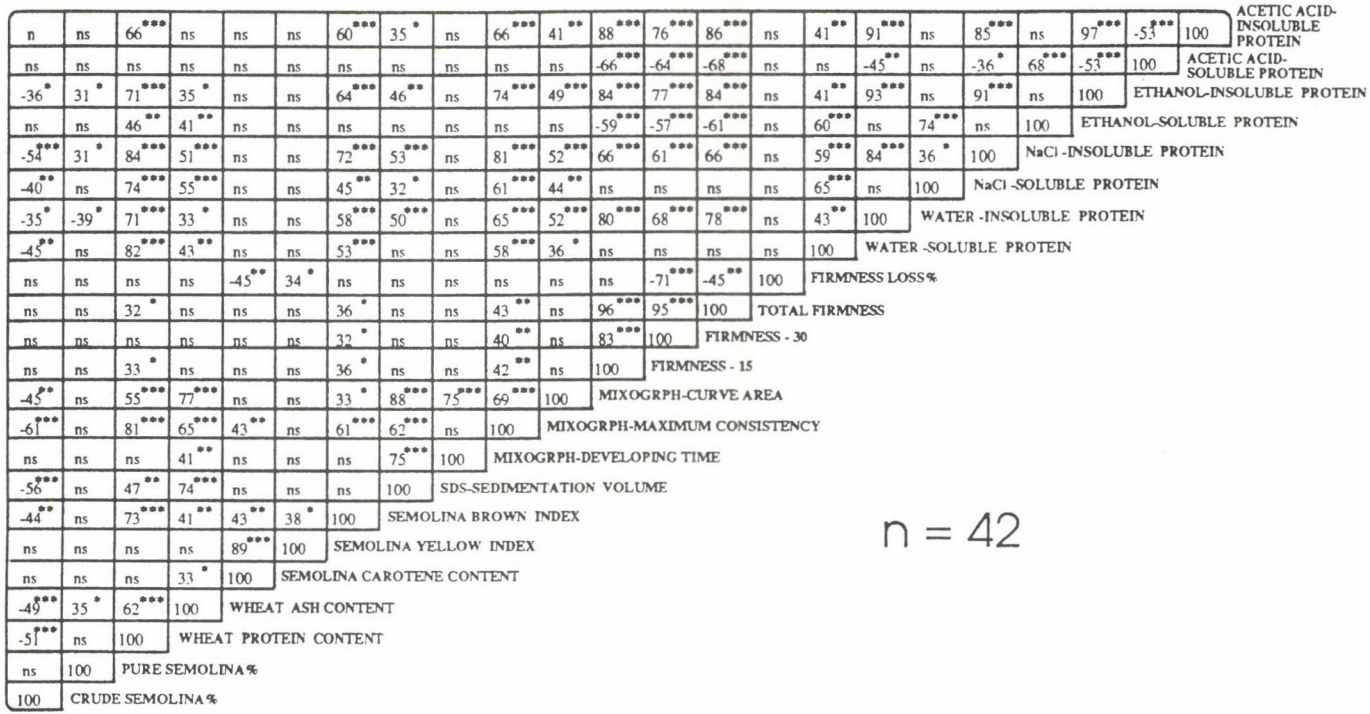

Fig. 1. Correlation matrix of 23 durum wheat quality variables (r×100)
***: very highly significant. (P<0.001). **: highly significant (P<0.01).
*: significant (P<0.05) and ns: not significant

2.3. Protein solubility fractions – cooking quality associations

According to the correlation coefficient values shown in the correlation matrix (Fig. 1), relating the insoluble-residue protein fractions and the cooking quality parameters, the most remarkable observations was that protein contents of the insoluble residue fractions isolated with water, 0.5 mol NaCl, 70% ethanol and 0.1 mol acetic acid were strongly associated with each other and showed very highly significant positive correlations with pasta cooking quality parameters, i.e. firmness of normally cooked pasta (15 min) (F15), its tolerance to overcooking up to 30 min (F30), and total alveograph value (FT). However, no correlation was found between the percentage of pasta firmness loss (FL%) caused by overcooking and any of the protein soluble fractions. These results confirmed previous reports stating that a significant quantity of higher molecular weight, particularly acetic acid-insoluble proteins, were necessary for good pasta quality (STEFANIS et al., 1990; GALTERIO et al., 1988, 1991, 1993). The acetic acid-insoluble protein of semolina has been suggested for predicting pasta cooking quality by SGRULLETTA and DE STEFANIS (1989).

3. Conclusions

From the new set of samples used in this study, the conclusive result was a very strong confirmation of the genetic basis of durum wheat quality parameters. Breeders must know that they can not breed efficiently for good cooking quality through increasing protein content and gluten strength without sacrificing good pasta colour. Both colour score and cooking quality must be improved separately. Pasta colour has been shown to be dependent on two parameters: brown index, which can be predicted through wheat ash and protein contents, particularly the insoluble protein fractions, and yellow index, which might be predicted by semolina carotene content, but with oxidative enzyme activities taken into consideration.

Improvement of pasta colour depending on genetic basis seems to be more difficult than cooking quality. However, other ways regarding inactivation of oxidative enzymes, i.e. with heat-treating (KATHURIA & SIDHU, 1984), gamma irradiation (TAHA, 1990) of wheat or semolina, and high temperature drying of pasta (DEXTER et al., 1981; WYLAND & D'APPOLONIA, 1982; STEFANIS et al., 1990) might be useful in this respect.

Total protein content and tests related to gluten quality (SDS-sedimentation volume and mixograph parameters) are not efficient to predict pasta cooking quality. Accordingly, breeders need fast, small-scale methods to predict the different parameters of cooking quality. Genetic improvements depend on their capacity to efficiently screen thousands of lines each year.

The results of this study suggest the possibility to develop a durum wheat index for predicting pasta cooking quality based upon the directly strong relationships between cooking quality parameters and insoluble-residue protein fractions, particularly those isolated with 0.1 mol acetic acid and 70% aqueous ethanol. Such an index requires only small amount of grain, and the technique should be applicable at relatively early generations of breeding programs for prediction of pasta cooking quality. A large scale study to verify the usefulness of this index is necessary.

An urgent need exists for research to identify other factors involved in the quality of durum wheat and to develop new testing procedures to complement those currently in use.

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STRUCTURAL AND OTHER ALTERATIONS IN PLANT TISSUES CAUSED BY QUICK-FREEZING AND STORAGE TIME

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The light and electron microscopy tests show that permanent histological and cytological changes take place after blanching, then after freezing and then in the first 2 to 3 months of storage. These changes can be characterised by the deformation of the parenchyma, the increase in the size of intercellular compartments and not too often by the occurrence of shrunk cells with thinning cell-walls and decreasing strength, and by smaller or larger ruptures at the older texture areas. During the storage period the texture of celery remains relatively stable. With the exception of the slow reduction of the lightness value the same applies to the colour properties of the product. The pectin content gradually decreases after freezing, the dripping loss however, increases slowly.

Keywords: celery root, cell structure, colour, freezing, frozen storage, texture

As far as the preservation of the original sensorial and nutritional properties of products is concerned industrial food freezing and frozen storage belong to the gentle preservation methods. Despite of this there are/can be some non-desirable permanent changes of biological, physical and chemical nature that may cause the deterioration of end product quality, modify shelf life and increase per unit material and energy costs.

Recent research, besides characterising the changes in the chemical composition of quick frozen and then stored products of plant origin, has been dealing, among others, with the effects of technological processes such as blanching methods, freezing without blanching, freezing rate, storage temperature and thawing conditions with regards to the preservation of the texture and colour of popular vegetables or to the changes in the structural condition of tissues and cells (FORNI et al., 1991; FUCHIGAMI et al., 1994; KIM & HUNG, 1994; FUCHIGAMI et al., 1995a,b). However, the utilisation of research results is made difficult by the fact that the optimal freezing rate is not known for vegetables and the publication of 1-year storage observations is relatively rare.

Our research work is mainly aimed at presenting in celery root the extent and possible consequences of raw material, technology and storage period-dependent biological (histological) changes and of other related changes.

1. Materials and methods

The raw material used in the experiments was field grown celery root (*Apium graveolens* L., Rapaceum group, cv. Imperator) and/or celery prisms (oblong shape) of industrial origin. The raw, cleaned (steam peeled) cut celery was blanched in water at 90–92 °C for 4 min then cooled down. Quick freezing took place in an industrial fluidised bed freezer with continuous operation down to a final temperature of −18 °C (air flow temperature was −28 °C and freezing time was 8 min). The celery was stored in polyethylene bags (30 μ thick), sealed by Hassia thermoforming sealing machine in units of 2.5 kg at −20 °C for 1 year. For the drip loss that characterises the whole technological process the product was thawed at room temperature (20 °C) in still air in 2 h. The same defrosting method was used for the other tests. Samples were taken in fresh condition (prior to processing), and then after blanching, 1 day after freezing, then on 14th, 55th, 107th, 195th and 365th days of storage.

The colour of the samples was objectively measured with Momcolor-D tristimulus colorimeter by reflection technique. The white enamel standard of the National Office of Measures, Hungary, No. 80-26-00 was used for comparison. Geometry of the measuring head was 0°/45°, optic angle 2°, CIE C illumination. Diameter of the diaphragm was 10 mm. Against the disturbing effect of outside light the sample was covered with a black cap.

For the objective description of product structure (using the Instron Universal Testing Machine, 4302) the size of the celery-prisms was 8 mm × 10 mm × 10 mm. The measurements were made at 20 °C. Load cell sensitivity was 100 N, crosshead speed was 10 mm/min, the sample rate of the measured points was 10 points/s.

The method used was measurement up to 75% compression. Characteristics: F_b (N): bio-yield point, the first peak on the curve; F/D (N/mm): coefficient of elasticity in response to compression, the slope of the linear section of the interval up to the bio-yield point (for frozen and stored sample since there is no bio-yield: the slope of the section belonging to smaller compression); F_{max} (N): force applied for 75% compression.

Relaxation method: compression up to the force of 2 N for 3 min. Characteristics: F/D (N/mm): coefficient of elasticity at relaxation, the slope of the linear section of the interval lasting until the emergence of the counter force of 2 N; β = relaxation factor measured after the elapse of 12 s; relaxation time (s): time needed for the reduction of force from 2 N to 1.5 N.

From the centre of raw, blanched and frozen celery-prisms, samples were cut in blocks of 125 mm^3. Tissue blocks were fixed (temperature of the fixation solution for frozen celery was −20 °C); post-fixed, dehydrated, embedded, cut and then stained for observation with a Nikon Laborphot-2 light microscope (LM) (ZACKEL, 1993). For

the conventional scanning electron microscope (SEM) tests samples of 5 mm×5 mm×2 mm were prepared from the centre of the celery-prism and they were fixed in buffered glutaraldehyde of 5% (for 2 h), then post-fixed in OsO_4 of 1% which was also buffered. Dehydration was made with a series of acetone, then with a critical-point dryer (Balzers CPD 020). The dry samples were glued on aluminium blocks and coated with Au-Pd layer. The SEM investigations were made with a Tesla BS 300 device at 20 kV.

For the determination of pectin fractions, following an alcoholic breakdown, a multiple extraction was carried out using distilled water, ammonium oxalate and 0.1 mol sodium hydroxide. The extracts were examined with spectrophotometry (Beckman DU-64, 525 nm) on the basis of colour reaction between galacturonic acid and carbazole.

Residual peroxidase-enzyme activity (POD, $E\ g^{-1}=1\times10^{-3}\ \Delta A\ min^{-1}\ g^{-1}$) was determined using o-phenylene-diamine hydrogen donor by spectrophotometry (Spektromom 195 D, 420 nm).

2. Results and discussion

Celery-prisms have a basic colour of yellow with greenish colouring and high lightness value. After blanching the green colouring increases and it can be demonstrated that the yellowness and the product's lightness decreases. During storage, and especially in the first six months, the colour properties L*, a*, and b* fluctuate to a small extent. Colour characters registered versus time show a weak relationship. In the final phase of the 1-year frozen storage the b* and L* values change significantly. On the basis of the latter the fading of the surface colour has an effect on the quality and it can be observed by the consumer (Table 1, Fig. 1).

The values of the rheological properties tested by compression method (F_b, F/D, F_{max}) decrease after blanching by 60 to 82% compared to fresh celery and this process remains characteristic even during freezing (Table 2, Fig. 2). In frozen celery the F_b peak disappears, the product's internal structure has no characteristic destruction point. In addition to the blanching process, therefore, also the freezing process contributes to the softening of products. In the later stage of frozen storage a relative stability can be observed on the macrostructural level. Similar tendency is indicated by the changes in the relaxation elasticity factor of celery-prisms (Table 3, Fig. 3). In Fig. 2 and 3 typical curves are presented that show the changes the best selected from ten measurements per treatment. Relaxation time greatly decreases as a function of technological treatment before storage. This reaction can be explained by the modified

water movement caused by the injury of cell walls and by the change of state of the space between cells. During storage no quality change takes place that could be statistically proven and termed as long lasting.

Table 1

Changes of the colour components of celery during processing and frozen storage

		a* Greeness	b* Yellowness	L* Lightness	C* Chroma	h° Hue angle
Raw	M	-3.16	13.24	80.74	13.62	-76.12
	±s	0.42	3.07	2.95	3.12	2.64
	CV	13.25	23.18	3.66	22.94	3.47
Blanched						
	M	-4.59^{c}	10.46^{a}	62.88^{bc}	11.54^{a}	-64.10^{b}
	±s	0.96	3.98	4.13	3.71	9.83
	CV	20.84	38.03	6.57	32.10	15.34
Freezing, frozen storage (day)						
1	M	-5.02^{ab}	9.71^{a}	60.71^{ab}	11.01^{a}	-61.27^{ab}
	±s	0.51	2.51	3.41	2.20	8.23
	CV	10.12	25.83	5.62	19.94	13.44
55	M	-4.59^{c}	10.46^{a}	62.17^{ab}	11.48^{a}	-65.22^{bc}
	±s	0.53	2.76	3.74	2.55	5.91
	CV	11.55	26.36	6.02	22.19	9.06
107	M	-4.91^{bc}	10.13^{a}	59.93^{a}	11.32^{a}	-63.05^{ab}
	±s	0.61	2.65	3.68	2.44	6.52
	CV	12.43	26.11	6.13	21.56	10.35
195	M	-5.39^{a}	9.32^{a}	61.78^{ab}	10.84^{a}	-58.90^{a}
	±s	0.60	2.68	3.18	2.45	6.56
	CV	11.09	28.71	5.15	22.59	11.13
365	M	-4.71^{bc}	12.89^{b}	65.05^{c}	13.79^{b}	-69.08^{c}
	±s	0.73	3.17	3.34	2.97	5.80
	CV	15.46	24.56	5.13	21.57	8.40
	LSD	0.42	1.88	2.25	1.73	4.56

^{a,b,c}: Mean separation in columns (without "Raw") by Duncan's multiple range test, as $P<0.05$ level, ($n=20$)

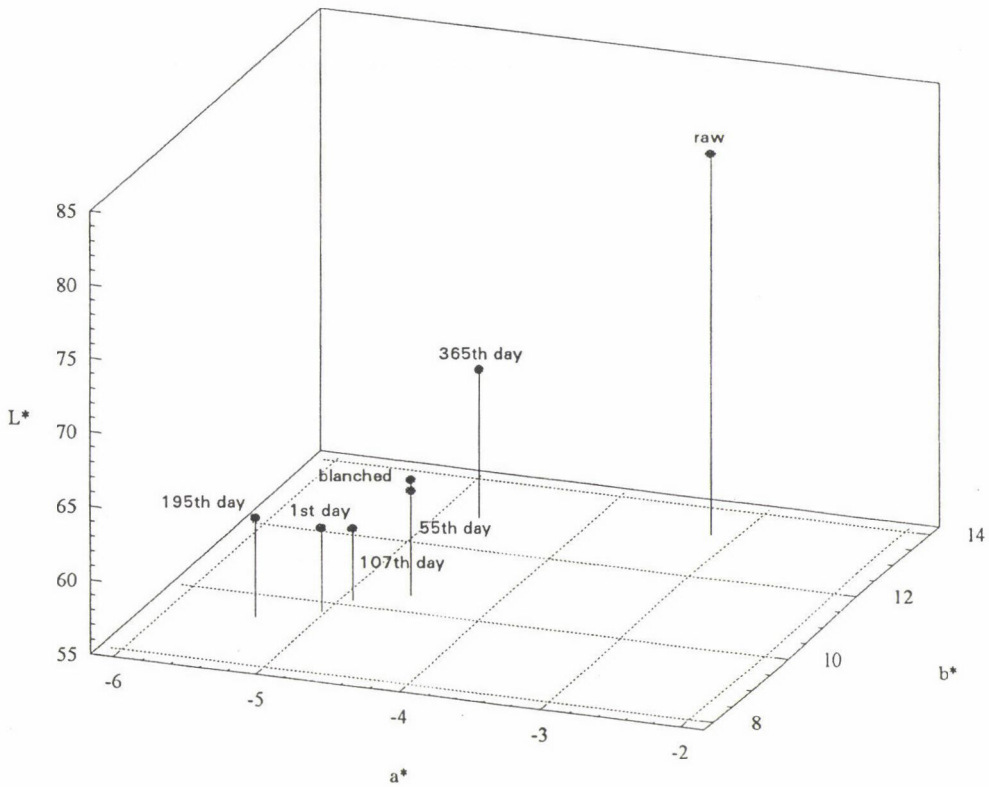

Fig. 1. Colour points of raw and processed (blanched, frozen) celery in CIELAB colourspace

The light and electron microscope tests show that prior to the processing operation fresh and raw celery is characterised by thin-walled cells in which tissue sections with loose and thick texture, with older large cells and younger small cells alternate (Fig. 4). Blanching, which cannot be avoided from technological point of view, has smaller effect than expected. The denaturing of the cytoplasm, the damage of the thin walled cells is in fact minimal.

Table 2

Development of texture characters of celery tested by compression method with INSTRON

		F/D (N/mm)	F_b (N)	F_{max} (N)
Raw	M	44.47	120.56	168.51
	±s	8.67	20.26	29.91
	CV	19.50	16.80	17.75
Blanched	M	8.18	48.70	58.32
	±s	2.58	10.81	18.50
	CV	31.57	22.19	31.72
Freezing, frozen storage (day)				
1	M	1.06^{ab}		33.93^{ab}
	±s	0.20		7.56
	CV	18.79		22.27
14	M	1.10^{ab}		37.02^{b}
	±s	0.22		6.07
	CV	19.81		16.40
55	M	1.07^{ab}		35.53^{ab}
	±s	0.32		9.97
	CV	29.81		28.06
107	M	1.18^{ab}		35.47^{ab}
	±s	0.22		10.94
	CV	18.42		30.85
195	M	1.21^{b}		28.60^{a}
	±s	0.25		6.55
	CV	20.97		22.90
365	M	0.93^{a}		34.59^{ab}
	±s	0.35		12.73
	CV	37.77		36.81
	LSD	0.24		8.53

F/D: coefficient of elasticity, F_b: bioyield point,
F_{max}: force applied for 75% compression
^{a,b,c}: Mean separation in columns (without "Raw" and "Blanched") by Duncan's multiple range test, as $P<0.05$ level, ($n=10$)

Table 3

Trend of texture characters of celery measured by relaxation test with INSTRON

		F/D (N/mm)	β-factor	Relaxation time (s)
Raw	M	10.94	0.16	42.29
	±s	1.40	0.07	26.58
	CV	12.83	43.66	62.84
Blanched	M	3.74	0.37	4.99
	±s	0.64	0.08	3.09
	CV	17.23	23.01	61.85
Freezing, frozen storage (day)				
1	M	0.98[a]	0.70[a]	0.46[a]
	±s	0.38	0.06	0.27
	CV	39.10	8.88	58.99
14	M	1.13[a]	0.69[a]	0.50[a]
	±s	0.38	0.04	0.12
	CV	33.41	5.77	24.07
55	M	1.11[a]	0.66[a]	0.52[a]
	±s	0.31	0.05	0.21
	CV	28.35	7.64	39.81
107	M	0.67[b]	0.69[a]	0.43[a]
	±s	0.27	0.03	0.20
	CV	40.54	4.92	46.86
195	M	1.10[a]	0.68[a]	0.57[a]
	±s	0.32	0.03	0.13
	CV	29.24	4.49	23.38
365	M	1.05[a]	0.41[b]	0.58[a]
	±s	0.38	0.04	0.19
	CV	35.91	10.26	32.51
	LSD	0.29	0.04	0.18

F/D: coefficient of elasticity

[a,b,c]: Mean separation in columns (without "Raw" and "Blanched") by Duncan's multiple range test, as $P<0.05$ level, ($n=10$)

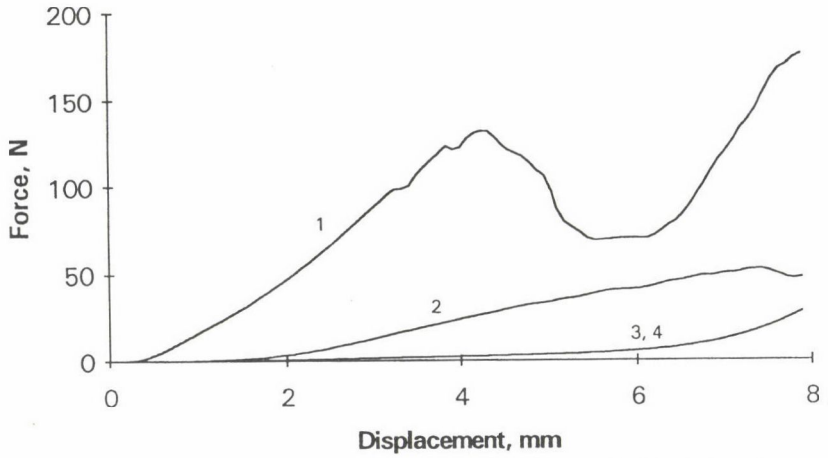

Fig. 2. Typical compression curves of raw and processed celery. 1: raw; 2: blanched; 3: frozen storage, 1st day; 4: frozen storage, 365th day

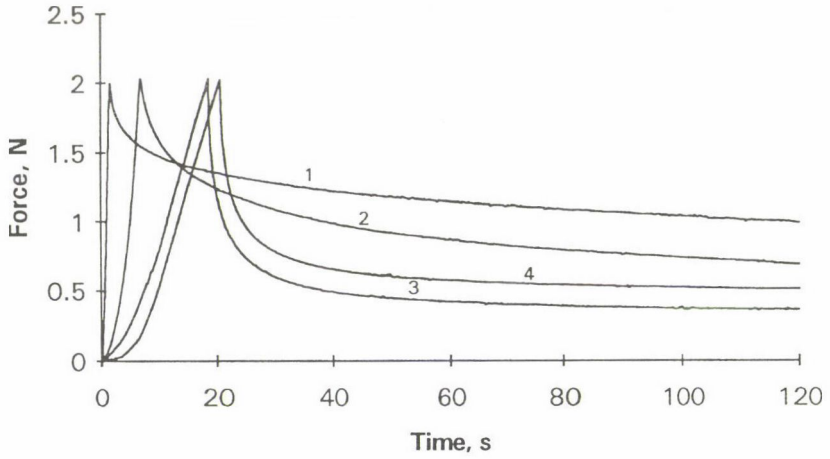

Fig. 3. Typical relaxation curves of raw, blanched and frozen celery. 1: raw; 2: blanched; 3: frozen storage, 1st day; 4: frozen storage, 365th day

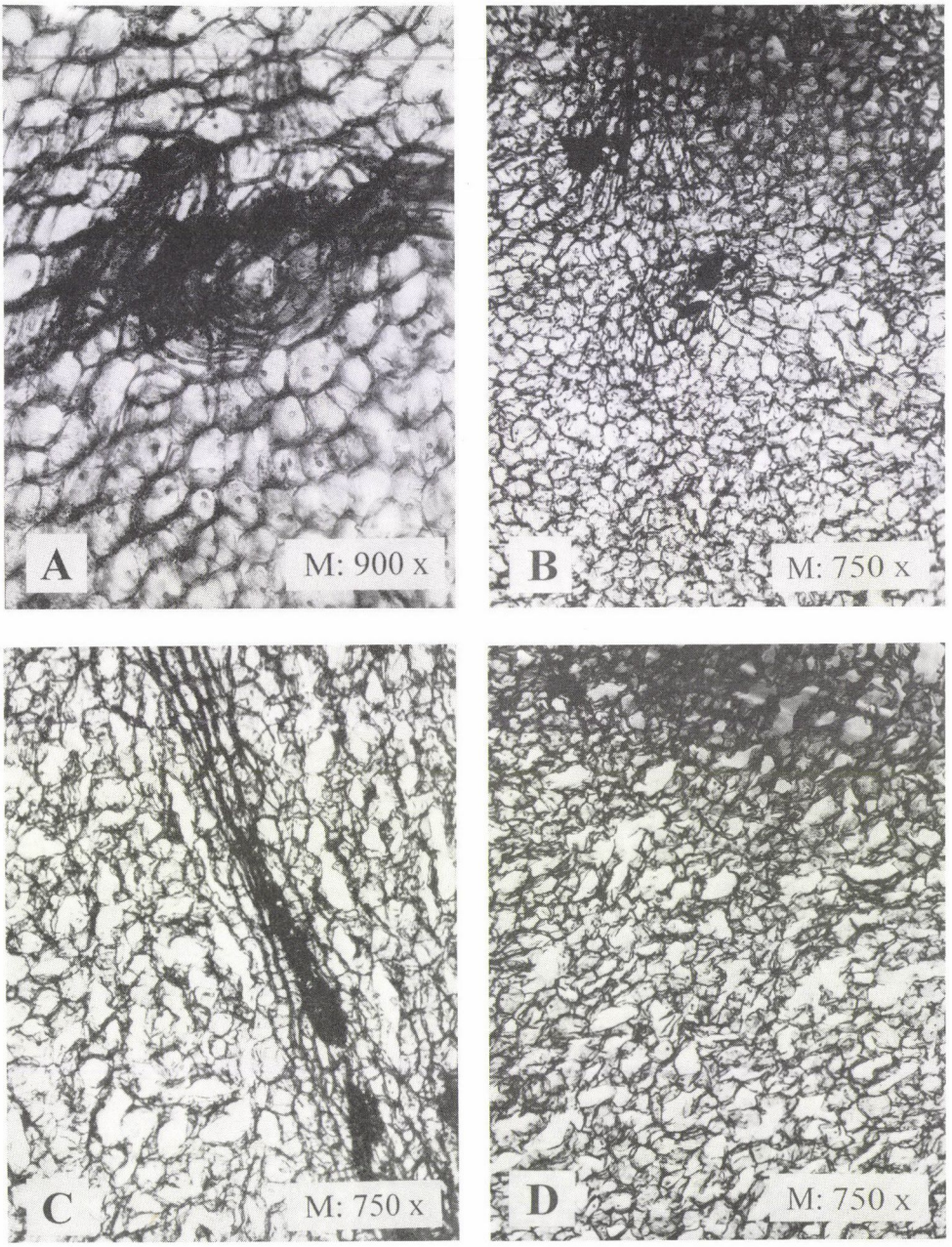

Fig. 4. Light micrographs showing parenchyma of raw and processed celery root. A: raw celery; B: 1 day after freezing (blanched for 4 min); C: 195th day of frozen storage; D: 365th day of frozen storage

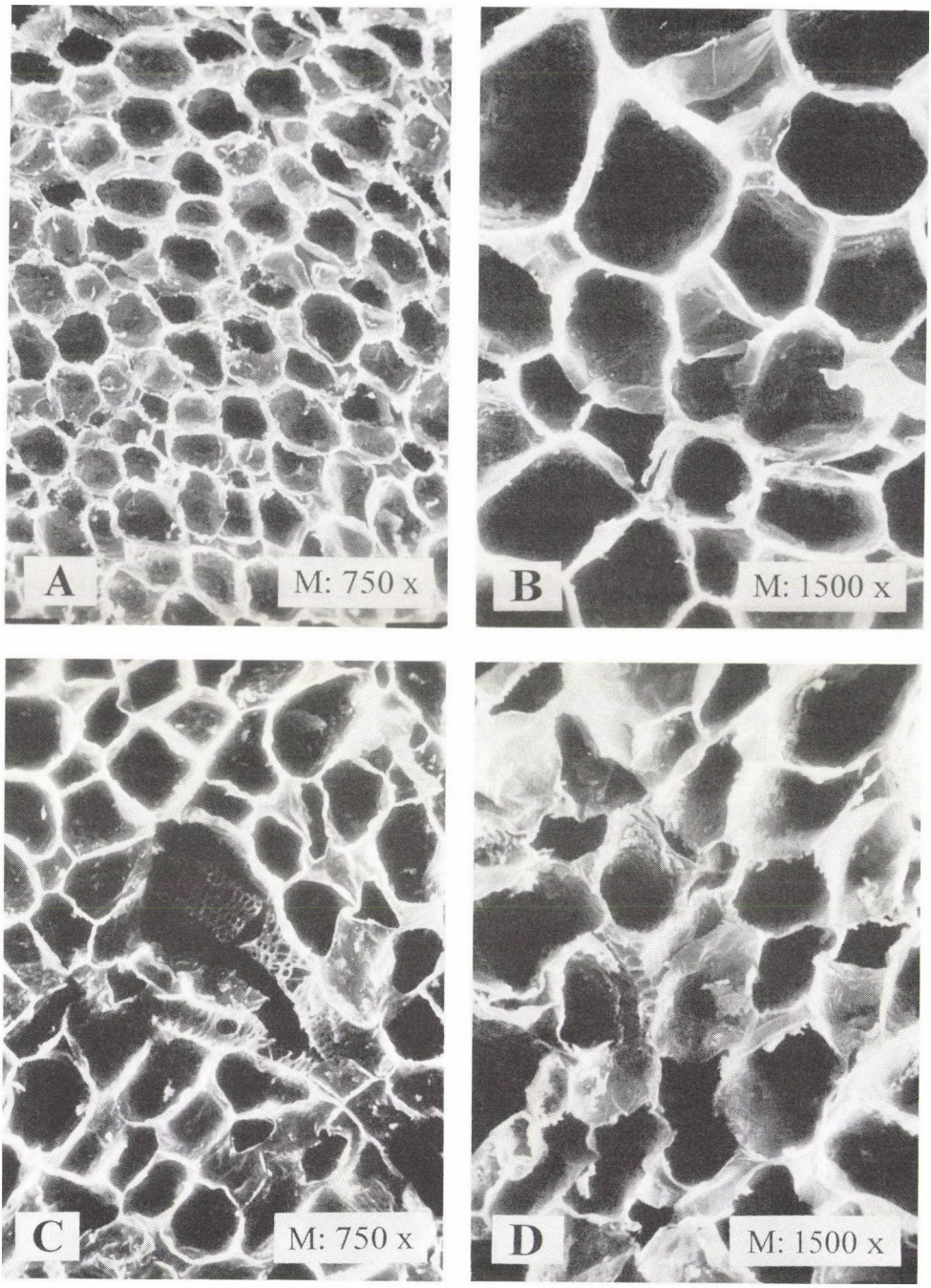

Fig. 5. Conventional scanning electron micrographs showing parenchyma of raw and processed celery root. A: raw celery; B: 1 day after freezing (blanched for 4 min); C: 195th day of frozen storage; D: 365th day of frozen storage

During quick freezing (or rather one day later) in the heterogeneous tissue cavitation occurs due to the separation of cells. The micelles consisting of cellulose molecules are squashed and some loosening takes place in the cell wall. That part of the product, however, which is made up of supporting or mechanical tissue (relationship of the prepared celery sample to the original root) changes its structure in a smaller degree than it can be expected (Fig. 5). During storage the process of damage to the cell wall and the increase of the size of intracellular compartments continues moderately, but the change of shape of cells, e.g. shrinkage hardly occurs. The above mentioned changes are more moderate along the transport vascular bundles. In spite of these alterations the histological structure of the celery can be satisfactorily preserved during storage.

Freezing in bulk is regarded by the industry and trade as a quality deteriorating factor causes some tissue damage but it is probable that the danger does not lie here but in the local desiccation occurring in the tissue structure of product areas in contact with one another and in the effect of this.

Total pectin content of the celery-prisms at the start of processing is 48 mg/100 g product. After freezing process the loss, including blanching, is 10%. During the 1-year storage the degradation of pectin is relatively gradual and moderate. Total loss is 23%, of which 13% falls to the storage period. Pectin content in the second period of storage is significantly lower compared to the value measured directly after freezing, and its effect is manifested on cell wall damage (Fig. 6).

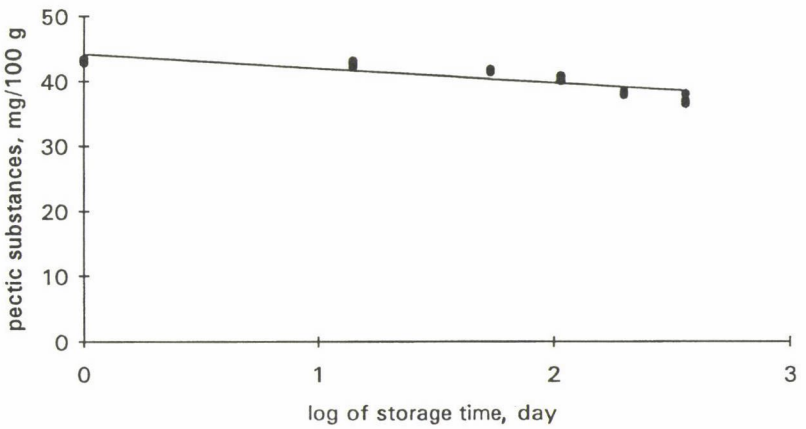

Fig. 6. Result of regression analysis of celery pectic substances changes during frozen storage. $Y'=44.12-2.19 \log t$; $r=-0.861$; $n=24$

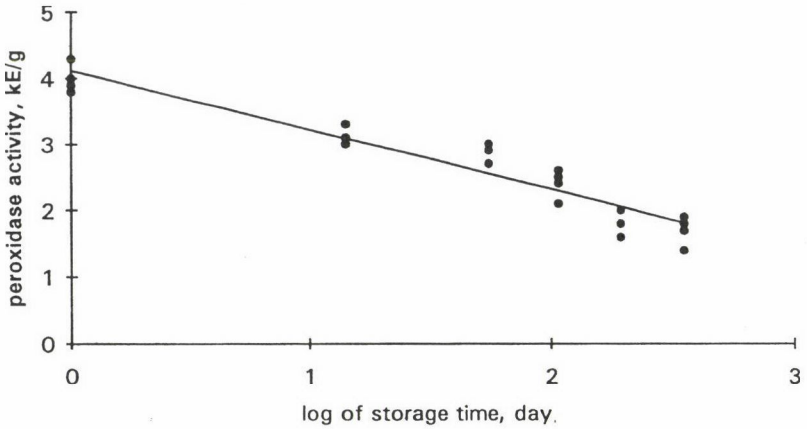

Fig. 7. Linear regression analysis of the alteration of peroxidase activity of celery during frozen storage. Y'=4.12–0.90 log t; r=–0.970; n=24

Peroxidase, also known as an indicator enzyme, found partly in the cytoplasm and partly bound to the cell wall, shows a reduction in activity of 24% during blanching, 2% during freezing and a total of 58% during the 1-year storage period (Fig. 7). POD activity shows a relatively lower level during second half of the storage period (FRETZDORFF & BERGTHALLER, 1989). All these explain why the external and internal quality of the product can be maintained for a long period of time.

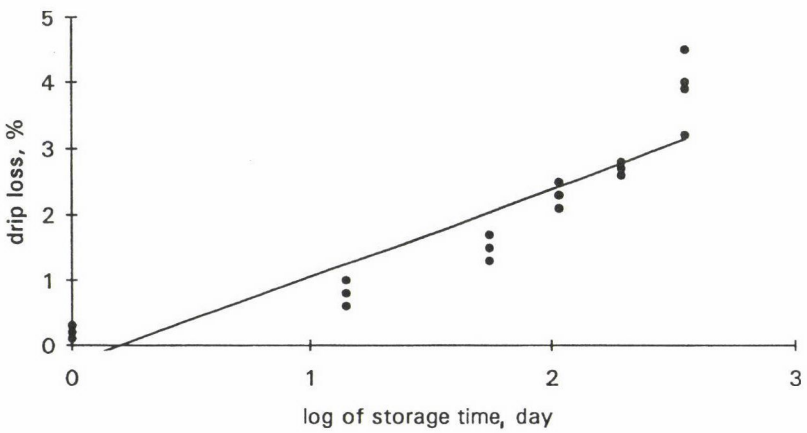

Fig. 8. Linear regression analysis of the development of drip loss of celery during frozen storage. Y'=–0.27+1.33 log t; r=0.924; n=24

Drip loss related to the necessary thawing shows a slow increase but stays at an acceptable level during the 1-year period (0.2–4.5%) considering both industrial and consumer's demands, proving that the histological and cytological stability and the satisfactory texture are coupled with good water retention ability during storage (Fig. 8).

3. Conclusions

Our tests showed that quick-frozen celery-prisms show important changes of physical, biological and chemical nature mainly following the blanching and freezing processes. However, the length of storage period do not greatly influence the satisfactory texture of celery, its colour, an histological state. The moderate loss of materials also includes the degradation of some of the pectins. The phenomenon of bulk freezing occurring during storage induces slight structural changes but these do not affect the acceptance of the product by the consumers.

*

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Short communications

EXTENDING THE SHELF-LIFE OF CITRUS FRUITS USING IRRADIATION AND/OR OTHER TREATMENTS I. "BALADY" ORANGES*

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Abstract

Irradiation process (0, 1.50 and 2.50 kGy) with or without other treatments before irradiation, i.e. soaking in $CaCl_2$ solution or waxing were used in this study to investigate the effect of such treatments on the shelf-life of "Balady" orange fruits at room temperature.

Marketable properties (browning, decay and texture) in addition to the organoleptic evaluation of firmness, appearance, odour, colour and taste were detected. Results showed the preferability of waxing treatment before irradiation processes.

On the other hand, statistical analysis of the organoleptic evaluation revealed that the shelf-life of untreated sample (control) was 20 days at room temperature, while samples exposed to the different suggested treatments were rejected after 30 days under the same conditions.

Introduction

Ionizing radiations can performe four distinct functions in food processing depending on the dose of radiation applied and the type of food exposed:

- Preserve food by destroying the microorganisms, that cause normal spoilage.
- Kill the food-borne parasites and pathogenic microorganisms.
- Slow down the ripening of some fruits and inhibit the sprouting of others such as potatoes and onions.
- Sterilize or kill the adults, larvae and eggs of insects.

It must be noted that irradiation is not suitable for all foods and it cannot be used to improve inferior products to better one. However, the amount of radiation dose

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received by food items is critical because, too low dose will not produce the desired effect and too high dose may create undesirable changes in flavour and texture.

The World Health Organization (WHO) and International Atomic Energy Agency declared in 1983 that irradiation up to 10 kGy is the safe technological treatment. In 1985, the Federal Food and Drug Administration (FDA) made a general clearance for the irradiation of fresh foods to a maximum (1 kGy) to kill insects and inhibit growth, ripening and spoilage of fruits and vegetables (AECL, 1986).

In this study trials are considered to extend the shelf-life of "Balady" orange fruits using irradiation doses alone or in combination treatments at room temperature.

Materials and methods

Mature Balady oranges "Citrus sinensis cv. Balady" were purchased from El-wady Company for Exportation of Agricultural Products, Kaloubia Governorate, Egypt. The following treatments were carried out:

1. Control (untreated) (B)
2. Soaking in $CaCl_2$ solution, 50 ppm at 50 °C for 5 min (S)
3. Coating with thin layer of wax (W)
4. Irradiation with 1.5 kGy (B+1.5 kGy)
5. Irradiation with 2.5 kGy (B+2.5 kGy)
6. Irradiation with 1.5 kGy after soaking treatments (SB+1.5 kGy)
7. Irradiation with 2.5 kGy after soaking treatments (SB+2.5 kGy)
8. Irradiation with 1.5 kGy after waxing treatments (WB+1.5 kGy)
9. Irradiation with 2.5 kGy after waxing treatments (WB+2.5 kGy)

Samples were transported to the National Centre for Radiation Research and Technology (NCRRT) at Nasr City, Cairo, Egypt where the irradiation treatments were applied using cobalt-60 facility "Egypt Mega Gamma 1" model AECL 6500, the dose rate being 12 rad/sec. All treated samples were stored at room temperature (20–25 °C) and at a relative humidity of 72–74% in carton boxes of 6 kg oranges (usually used as export package unit).

The number of browned and decayed fruits were calculated during storage period and divided by the total number of fruits to obtain its percentages.

Instron Model 1140 (USA) was used to measure the texture of fruits at four locations under the following conditions: speed chart, 100 m/min; drive speed, 100 m/min; force range 10 kg and values of peak force range was calculated as kg/cm^3.

Firmness, appearance, odour, colour and taste were organoleptically evaluated. Values were statistically analysed according to SNEDECOR and COHRAN (1967) through the computer programs (Statistical Analysis System, SAS, 1979 and 1981).

Results and discussion

Marketable properties

Browning and decay. The treatment of 2.5 kGy gave the highest percentage of browned fruits whether soaked in $CaCl_2$ before irradiation or not. Same findings were reported by KUROSAKI and OGATA (1971), OJIMA and co-workers (1974), BELLI-DONINI and BARALDI (1977) and MOHAMED and co-workers (1989). The level of browning as number of defected fruits were 86 and 85% for B+2.5 kGy and SB+2.5 kGy, respectively. This trend may be related to the respiration phenomenon of the fruits in addition to the increment of permeability of the epidermal tissues of the irradiated samples. OJIMA and co-workers (1974) found that browning defect in citrus peel caused by irradiation was greater at 1.5 kGy and occurred sooner at higher storage temperature (20 °C). At the end of storage, the level of browning in the investigated samples increased.

After 15 days of storage, the dose of 2.50 kGy caused higher percentage of decay (11%) when compared to other treatments (1–4). FAROOQI and co-workers (1987) reported that irradiation process increased respiration and ethylene production in citrus fruits, and the degree of injury during storage significantly increased with radiation dose, too.

On the other hand, the 1.5 and 2.5 kGy treatments caused higher percentages of decay (18 and 15%) comparing to the combination treatments that recorded minimal percentages (2 to 6%) at the end of storage period. These results are in agreement with the results of browning. So, the preferability of waxing treatment could be noticed. This trend may be due to the prevention of evaporation as well as covering the injured surface and protecting the fruits against microorganisms. Concerning the medium values of decay low doses of irradiation inhibit the infection of orange fruits by microorganisms. For the high values of decay that is occurred due to high evaporation and transportation, consequently shrinkage of the surface and rotten of fungi which highly occurred at the end of storage period. Such values were also observed in fruits soaked in $CaCl_2$ this is may be due to that Ca-ions activate the lipid oxidation which plays an important role in browning of some fruits (FARAG, 1989).

Texture. The unirradiated fruits became softer than irradiated samples and the texture of the latter one was rapidly affected within one month of storage. However, no differences were observed among treatments at zero time of storage. 2.5 kGy

treatment caused a noticeable softening after 10 days of storage, while 1.5 kGy did not lead to any changes within the same storage period. Higher irradiation doses enhanced the degree of softness in relation to the control samples. It was 53% after 20 days in case of 2.5 kGy, however all treatments were discarded after 20 days. The best treatment was 1.5 kGy without combined treatments.

The role of waxing or calcium is to minimize texture loss due to high doses of irradiation up to 20 days. These results are in agreement with NAGAI and MOY (1985) and URBAIN (1986) who reported that softening which occurred at higher storage temperature and increasing dose is related to the irradiation degradation of the carbohydrates (cellulose, pectin and starch) associated with normal texture. Such degradation can affect the texture at least in two ways:

- by weakening the rigid structural tissues,
- by altering cell walls to reduce turgor.

A third possibility is affecting the process of endogenous enzymes either by releasing the enzymes from their normal locations into the plant tissue generally to where they can attack carbohydrate substances so as to make them more susceptible to enzyme action.

Organoleptic evaluation

The available data proved that, after 20 days of storage there is no significant difference between treatments in firmness. On the other hand, there is a significant difference in appearance between control sample and both (B+2.5 kGy) as well as waxed - irradiated samples. In addition, there is a significant difference between appearance of (S) and (SB+2.5 kGy) samples. Similar findings were found with waxed samples (irradiated or not). A significant difference in odour was recorded between control sample and (SB+2.5 kGy), as well as (WB+2.5 kGy) treatments. No reversible trend was found with other tested samples.

Regarding sample's colour, there is a significant difference between control and waxed sample in addition to the difference found between waxed samples (W, WB+1.5 kGy and WB+2.5 kGy). Statistical analysis of the taste parameter showed no significant difference between unirradiated samples and (B+1.5 kGy) treatment. On the other hand, a significant difference between (S) and (SB+2.5 kGy) as well as between (W) and (WB+2.5 kGy) sample was also detected. It is known, that the major discrepancies were found in appearance, flavour, taste and odour, they are less extreme at low dose. The previous data proved the preferability of waxing treatments as well as soaking treatment.

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AUTOMATED DOSIMETRY USING RADIATION SENSITIVE FILMS AND VALIDATION OF ROUTINE DOSIMETRY DATA*

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(Received: 13 February 1996; accepted: 6 March 1996)

Summary

A commercially available colorimeter was modified and connected to a PC. The usual cuvette holder was replaced by a motor driven, PC-controlled device for dose meter film handling. This modification enables reading of long strips useful for depth dose measurements or similar applications and of larger numbers of individual smaller pieces useful for dose mapping. Data are locked with the PC for later evaluation and graphical presentation.

Introduction

Several routine tasks in dosimetry consist of collecting and interpreting larger amounts of readings from series of dose meters and validating irradiation treatments. Upon commissioning of a new facility or after source replenishment or geometry modification usually extensive dose mapping is indispensable; during routine processing series of control measurements at several reference positions are generated. A reader with automatic dose meter handling and data locking could facilitate such tasks considerably. As a first step, the task of dose mapping and depth dose distribution measurement was tackled.

Approach

Dosimetry films have several advantages over other types of dose meters in routine application: They are rudged and low-cost, easy to handle, stable for prolonged pre-irradiation storage and suitable read-out periods after use, (nearly) independent of dose rate or radiation quality, allowing for spacial resolution, thin enough to be placed

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0139-3006/96/$ 5.00

on or in the goods and available as single systems for the full dose range of food irradiation (i.e. 10 Gy to 50 kGy). Such properties make them superior over several established routine systems, especially the PMMA-group which is most common in radiation sterilization.

Materials and methods

GAFchromic films (International Speciality Products, Wayne, NJ, USA) are suitable dosimetry materials in the dose range applicable for food irradiation (10 Gy to 50 kGy). Type DM-100 is a 10 mm wide film on a roll of 15 m. The photometric reader (Colorimeter 257, CIBA Corning Analytical, Halstead, Essex, England) allows for dedicated filters (interference, 405 nm). A film handling device fitting into the place of the cuvette holder was manufactured by the workshop and adapted for computer control by the electronics workshop (Fig. 1). The programmes detect whether a new film is inserted, start the measurement procedure, step the film through the optical reading zone and records the optical density values. Data are evaluated further by the use of SAS (SAS Institute Inc., Cary, NC, USA), and statistics and graphics are generated.

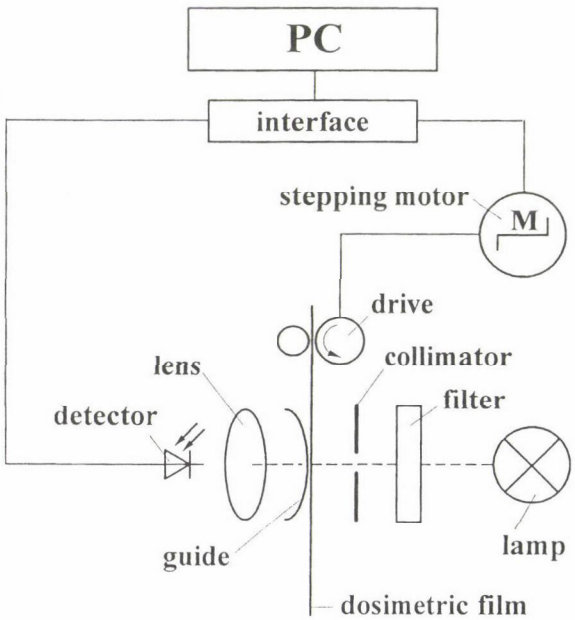

Fig. 1. Automatic reader for dose meter films (schematic diagramme)

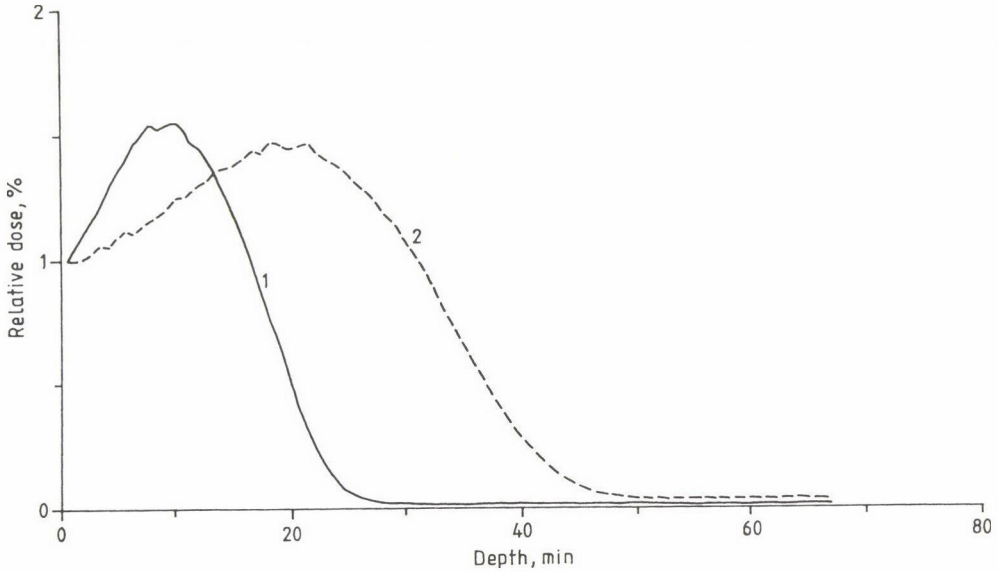

Fig. 2. Depth dose curves for 5 and 10 MeV-electrons, standardized for unit density and 100% entrance dose. Energy 1: 5 MeV; 2: 10 MeV; material: PVC

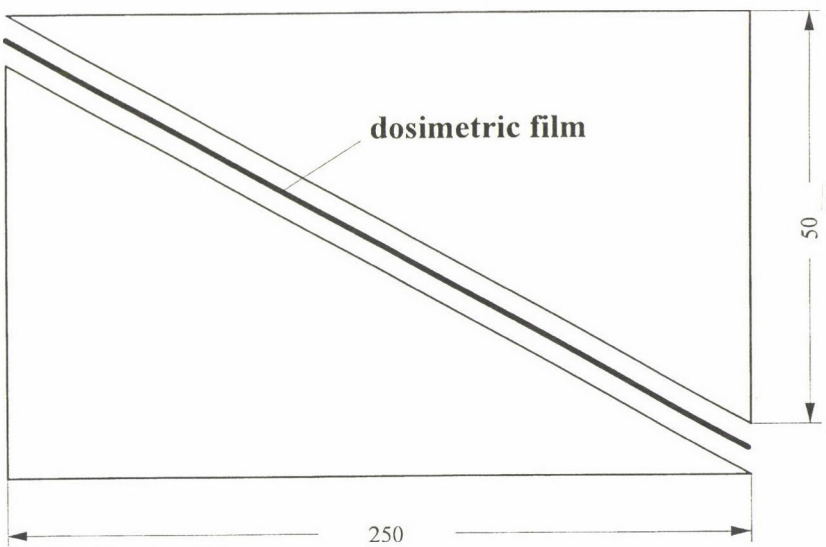

Fig. 3. Wedge for depth dose measurements (dimensions in mm)

Results and discussion

Facilitation of troublesome and tiring routine jobs greatly contributes to the overall quality of such business. Compiling of depth dose curves from individual dose meters placed at appropriate positions throughout the entity under consideration is such a boring task. Strategical placing dose meter strips and subsequent automatic reading and data processing is an adequate solution. As an example depth dose curves for 5 and 10 MeV electrons in water are shown (Fig. 2) which were produced using a pair of wedges with the film inserted (Fig. 3). Future modification of the film entrance mechanism shall allow for automatic reading also of larger number of clipped, small film pieces simply by pouring them on a vibrating tablet on top of the feeding mechanism.

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IONIZING RADIATION PROCESSING OF SOME FRESH FRUIT AND VEGETABLE*

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Fresh fruit and vegetables are very important from nutritional point of view, in Romania, having mainly temperate climate, the followings: apples, cherries, apricots, strawberries, tomato. The processing of these fruits in order to reduce the spoilage, the post-harvest losses and to extend their shelf-life is also important (DIEHL, 1990). For these purposes many techniques are known like: refrigeration, controlled atmosphere, chemical and fungicidal treatments, irradiation (THOMAS, 1986). These processes are applied either separately, or in combination.

The preliminary tests aimed at the demonstration of feasibility and efficiency of the irradiation processing of fresh foods of plant origin, as an alternative technique, both by γ-ray and electron-beam (FERDES et al., 1994).

Materials and methods

The fruits were: strawberries, cherries, sour-cherries, apricots, nectarines and apples, common cultivars. Tomatoes were also studied.

They were placed in mono-layer, in appropriate PE boxes, approximately 0.5 kg in each box and irradiated 18 h after harvest.

Pre- and post-irradiation storage conditions were: 4–10 °C temperature and 75–80% relative humidity (R.H.) and, in some cases, room temperature.

The irradiation was performed under normal conditions (20–22 °C, normal atmosphere, 70–75% R.H.) with a ^{60}Co γ-ray source and with a linear electron accelerator.

* Extended abstract of a poster presented at the Symposium on Current Aspects of Food Irradiation held in the frame of IUFoST 9th Congress of Food Science and Technology, 3 August 1995, Budapest, Hungary

0139-3006/96/$ 5.00

The ^{60}Co source had approximately 110 TBq (3,000 Ci) activity and a dose-rate of 3 kGy h^{-1} in a plane geometry. For dosimetry chemical (Fricke) dosemeter was used.

The accelerator main characteristics are: electron mean energy approx. 7 MeV, beam current 5 μA, pulse period 3 μs. Plane irradiation geometry was used with a 90° deflected electron-beam, at a dose rate range of 200–1000 Gy/min. For cheking the doses ionizing chamber-based dosemeters were used.

The irradiation doses varied for each food item, regardless to the source of irradiation (γ-ray and electron-beam irradiation).

The following parameters were investigated by the usual methods: shelf-life, organoleptic properties (size, shape, colour, taste, flavour), dry weight, total and reducing sugars, pH, conductivity, ascorbic acid content and so on.

All the reagents were of analytically pure grade.

Results and discussion

The main results are shown in Table 1 (a–g). The shelf-life extension for all the irradiated samples is emphasized.

The most efficient doses for the different fruits were: 2–3 kGy for strawberries; 1 kGy for cherries; 0.5–1.0 kGy for sour-cherries; 0.5–0.7 kGy for apricots; 1–2 kGy for nectarines; 0.5 kGy for apples (Jonathan cultivar) and 1.0 kGy for tomato.

The irradiation has no significant effect on the organoleptic properties if the appropriate irradiation dose is respected.

There are no significant changes in the physical and chemical parameters of the irradiated fruits with the very important exception of the ascorbic acid (vitamin C) content.

The ascorbic acid content in irradiated samples is significantly lower than in the non-irradiated fruits, but during storage the vitamin C losses are also lower in irradiated samples than in non-irradiated ones.

The other physical and structural changes could be related to the biochemical and functional changes, but, to elucidate these aspects more extended experiments have to be carried out.

In any case it could be concluded that the irradiation is feasible, both technologically and economically, an alternative to the other "classical" processes, and in some situation the irradiation could be successfully used for shelf-life extension and loss reduction of fresh temperate fruits and vegetables like strawberries, cherries, sour-cherries and tomatoes.

Table 1

Characteristic parameter values for irradiated foods of plant origin

1 a. Strawberries

Parameter		Irradiation dose (kGy) 0	1.0	2.0	3.0
		Shelf life (day) 3	5–7	7–10	7–10
Organoleptic properties	-at 3 days	0	++	+++	+++
	-at 5 days	–	+	+++	+++
Dry weight (°Brix)		7.80	8.50	9.40	9.30
Total sugar (% at d.w.)		66.7	66.6	76.7	71.1
Reducing sugar (% at d.w.)		66.7	60.0	59.4	52.1
pH value		3.68	3.70	3.75	3.75
Conductivity (μS cm)		375	360	340	308
Vitamin C (mg/100g)		23.2	22.9	21.7	20.8

1 b. Cherries

Parameter		Irradiation dose (kGy) 0	0.5	1.0	2.0
		Shelf life (day) 5–7	7–10	10–15	7–10
Organoleptic properties	-at 5 days	0	++	++	++
	-at 10 days	–	0	++	+
Dry weight (°Brix)		20.0	21.0	21.6	20.5
Total sugar (% at d.w.)		67.7	66.5	63.7	71.2
Reducing sugar (% at d.w.)		67.0	64.5	62.6	70.4
pH value		4.32	4.30	4.34	4.34
Conductivity (μS cm)		300	360	450	385
Vitamin C (mg/100g)		6.61	6.20	6.10	5.98

1 c. Sour cherries

Parameter		Irradiation dose (kGy) 0	0.5	1.0	2.0
		Shelf life (day) 5–7	10–15	10–15	7–10
Organoleptic properties	-at 5 days	++	+++	++	++
	-at 10 days	–	++	++	0
Dry weight (°Brix)		14.5	14.3	14.0	12.2
Total sugar (% at d.w.)		68.4	67.5	65.5	68.3
Reducing sugar (% at d.w.)		54.3	57.6	55.0	58.0
pH value		3.67	3.65	3.66	3.67
Conductivity (μS cm)		650	680	700	650
Vitamin C (mg/100g)		18.6	17.8	12.5	6.6

1 d. Apricots

Parameter		Irradiation dose (kGy) 0	0.5	0.7	1.0
		Shelf life (day) 10–12	15–20	15–20	10
Organoleptic properties	-at 5 days	++	+++	+++	++
	-at 10 days	–	++	++	0
Firmness (x 10^5 N m^{-2})		2.8	1.3	1.5	1.7
Dry weight (%)		16.2	16.9	18.5	17.7
Total sugar (% at d.w.)		50.6	46.3	51.6	45.7
Reducing sugar (% at d.w.)		48.2	42.9	47.9	45.6
pH value		3.27	3.16	3.27	3.25
Acidity (g citric acid/100 g)		2.279	2.408	2.448	2.230

1 e. Nectarines

Parameter		Irradiation dose (kGy) 0	0.5	1.0	2.0
		Shelf life (day) 15	10–15	10–15	15
Organoleptic properties	-at 7 days	+++	+	++	+++
	-at 15 days	+	–	+	++
Firmness (x 10^5 N m^{-2})		6.6	4.1	4.0	5.2
Dry weight (%)		13.0	11.9	10.8	10.8
Total sugar (% at d.w.)		55.6	56.5	51.0	53.2
Reducing sugar (% at d.w.)		50.6	50.7	47.9	48.6
pH value		3.80	3.92	4.01	3.89
Acidity (g citric acid/100 g)		0.844	0.872	0.810	0.614

1 f. Apples (Jonathan cultivar)

Parameter		Irradiation dose (kGy) 0	0.1	0.2	0.5
		Shelf life (day) 90–120	90–120	120–150	120–180
Organoleptic properties	-at 30 days	+++	+++	+++	+++
	-at 90 days	+	+	+++	++
Firmness (x 10^5 N m^{-2})		4.75	3.55	3.95	3.95
Dry weight (%)		17.5	15.6	15.2	15.2
Total sugar (% at d.w.)		13.5	12.2	11.7	12.0
Reducing sugar (% at d.w.)		9.1	9.1	9.1	9.0
pH value		3.73	4.06	3.72	3.67
Conductivity (μS cm)		200	247	194	212
Acidity (g/l)		3.62	3.49	4.01	4.60

1 g. Tomato

Parameter		Irradiation dose (kGy) 0	0.5	1.0	2.0
		Shelf life (day) 7–15	15–30	15–30	15–30
Organoleptic properties	-at 10 days	++	+++	+++	+++
	-at 30 days	–	+	++	0
Dry weight (°Brix)		5.5	6.5	5.0	5.7
pH value		4.65	4.59	4.63	4.55
Conductivity (μS cm)		480	560	560	630
Vitamin C (mg/100 g)		20.6	19.8	14.5	9.4

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RHEOVISCOMETRIC TECHNIQUE IN IDENTIFICATION, CONTROL AND QUALITY EVALUATION OF SOME IRRADIATED FOOD INGREDIENTS*

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The rheological behaviour of some starch-based food ingredients was studied after γ-ray and electron-beam irradiations, within the dose-range of 1.0–50 kGy (20% uniformity) at different dose rates. The shear stress (τ) and viscosity (η) of the gelified suspensions were analysed at different shear rates and $\dot{\gamma}$ irradiation doses, by using a rotational viscometer Rheotest 2, with co-axial cylinders S/S_2. The method pointed out a good correlation between the irradiation dose and the rheoviscometric properties, especially for $D > 1.0$ kGy and at shear rate $\dot{\gamma} > 27.0\ s^{-1}$.

It is well-known and widely accepted that, in comparison with other treatments, the irradiation of spices is effective to reduce the microbial contamination in order to provide the sanitation and quality standards, because spices, especially peppers and paprika are highly contaminated with microorganisms.

The consumers concerns, standardization and legal aspects require an efficient, precise and reliable system to check the compliances, to detect and to control the irradiated foods.

Therefore, considering the previous experience (FARKAS et al., 1990b; FORMANEK et al., 1994; HAYASHI et al., 1994), the rheoviscometric behaviour of corn starch, black pepper and paprika was studied at γ-ray and electron-beam irradiation. This study aims to clarify some aspects concerning ionizing radiation effect on starch damage, too. The viscosities (η) and the shear stress (τ) measurements have been proposed (CASANDROIU et al., 1994; FARKAS et al., 1990a; SCHREIBER et al., 1994) as a method to identify the irradiated samples, particularly those foods which contain starch.

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1. Materials and methods

There have been irradiated samples of corn starch, black pepper, paprika, the last two items from free market.

The γ-irradiation was carried out with a ^{60}Co source having approximately 110 TBq (3,000 Ci) activity, and a dose rate of 3 kGy h^{-1}, with dose uniformity 20%, checked by chemical standard dosimetry procedures.

The electron-beam irradiation have been performed by a linear accelerator, with the following parameters: electron-beam mean energy 7 MeV; beam current 5 μA, pulse period 3 μs. In this case the doses have been checked by a dosemeter with ionization chamber.

The rheoviscometric measurements were performed using a rotational viscometer type Rheotest 2, with co-axial cylinders couple S/S_2, at different shear rates ($\dot{\gamma}$) in the range of 1.0–437.4 s^{-1}.

The samples consist of:

- corn starch suspensions, 4% concentration, gelified at 95 °C, measured at 25 °C, after an incubation period of 18–22 h;
- black pepper suspension, 3 g starch-equivalent/100 ml distilled water in 33% NaOH solution, adjusted to pH = 12.0, gelified and measured at 26 °C, after an incubation period of 20–23 h;
- paprika suspension, 15 g paprika/100 ml distilled water, gelified and incubated for 19–20 h then measured at 27 °C.

The dry weight was determined using an IR moisture analyser, and the damaged starch content was analysed colorimetrically, at 555 nm (FARKAS et al., 1990b).

All the reagents used were of analytical grade.

2. Results and discussion

The results of the rheoviscometric analyses are presented in the Tables 1–3, for the above-mentioned prepared samples. The results represent the mean value of three determinations.

As expected, the shear stress (τ) and the viscosity (η) depends both on shear rate ($\dot{\gamma}$) and on the irradiation dose (D).

It is observed that the rheograms τ ($\dot{\gamma}$, D) and η ($\dot{\gamma}$, D) are good for $\dot{\gamma} > 27$ s^{-1}, but there are some problems at the lower values of the shear rate. The shear stress function and curves are more consequent than the viscosity ones.

The irradiation effect could be detected by viscometric measurements for $D > 1$ kGy, in any case.

Table 1

Rheoviscometric parameters for gelified (at 95 °C) suspension of corn starch (concentration 4%)

Shear rate, $\dot{\gamma}$ [s^{-1}]	Irradiation dose, D [kGy]													
	0		1		2		4		10		16		20	
	Shear stress, τ [Pa] and viscosity, η [mPa s]													
	τ	η	τ	η	τ	η	τ	η	τ	η	τ	η	τ	η
1.0	8.15	8149	7.22	7226	7.75	7749	5.53	5535	2.09	2091	0.24	246	0.03	31
1.8	8.67	4817	6.91	3844	7.45	4151	5.75	3194	2.06	1144	0.39	222	0.09	51
3.0	9.68	3229	7.68	2562	8.39	2798	6.30	2101	2.39	799	0.64	215	0.43	143
5.4	10.76	1993	8.42	1560	9.90	1834	7.38	1367	2.61	484	0.83	153	0.55	102
9.0	12.60	1401	9.53	1059	11.87	1319	8.15	905	2.95	328	0.98	109	0.73	82
16.2	14.91	921	12.15	750	13.62	841	9.28	573	3.20	197	1.10	68	0.95	60
27.0	17.06	632	15.52	575	14.30	529	10.05	372	3.72	138	1.56	58	1.23	45
48.6	21.37	440	17.22	354	15.22	313	11.07	228	4.61	95	2.33	48	1.84	38
81.0	26.75	330	18.45	228	16.45	203	12.36	153	5.99	74	3.13	38	2.52	31
145.8	32.74	225	21.52	148	18.91	130	15.22	104	8.85	61	4.73	32	3.93	27
243.0	39.51	163	25.98	107	22.66	93	19.12	79	12.02	49	6.94	29	5.65	23
437.4	50.58	116	34.44	79	29.36	67	26.07	59	17.37	40	10.45	24	9.04	21

Table 2

Rheoviscometric parameters for black pepper suspensions

Shear rate $\dot{\gamma}$ [s^{-1}]	Radiation type	Irradiation dose, D [kGy]									
		0		5		10		16		32	
		Shear stress, τ [Pa] and viscosity η [mPa s]									
		τ	η	τ	η	τ	η	τ	η	τ	η
1.0	γ		123	1.6	1559	0.1	123	0.1	123	–	–
	e	0.1		3.4	3383	5.4	5331	3.4	3383	3.1	3073
1.8	γ		205	2.3	1264	0.3	171	0.3	171	–	–
	e	0.4		4.6	2460	6.9	3827	4.7	2631	4.1	2255
3.0	γ		226	2.8	923	0.4	144	0.4	144	–	–
	e	0.7		5.9	1968	8.6	2870	6.0	1989	5.0	1681
5.4	γ		171	3.5	649	0.6	114	0.6	103	–	–
	e	0.9		7.5	1389	10.6	1959	7.7	1424	6.6	1219
9.0	γ		144	4.1	458	0.7	82	0.6	68	0.1	7
	e	1.3		9.0	998	12.6	1401	9.5	1059	7.8	868
16.2	γ		99	5.2	323	0.9	57	0.7	46	0.4	23
	e	1.6		11.5	710	15.5	957	11.8	729	10.0	619
27.0	γ		80	6.2	230	1.4	52	0.9	34	0.7	25
	e	2.2		14.0	517	18.8	695	14.2	526	12.3	456
48.6	γ		62	8.1	167	1.9	39	1.3	27	0.9	19
	e	3.0		17.3	357	23.4	481	17.9	368	15.4	318
81.0	γ		53	10.5	129	2.5	31	1.5	19	1.6	19
	e	4.3		22.0	271	28.9	257	22.4	277	20.0	248
145.8	γ		45	14.3	98	3.7	25	2.6	18	2.2	15
	e	6.6		28.9	198	38.1	262	29.6	203	26.6	172
243.0	γ		39	19.3	80	5.5	23	4.1	17	3.2	13
	e	9.3		37.6	155	48.3	199	38.1	157	34.7	143
437.4	γ	15	33	27.4	63	9.1	21	6.6	15	5.4	12
	e			52.3	120	66.0	151	52.6	120	48.6	111

Table 3

Rheoviscometric parameters for paprika suspensions

Shear rate $\dot{\gamma}$ [s^{-1}]	Radiation type	Irradiation dose, D [kGy]							
		0		5		10		20	
		Shear stress, τ[Pa] and visocisty, η [mPa s]							
		τ	η	τ	η	τ	η	τ	η
1.0	γ	0.7	738	0.6	615	0.25	246	0.3	307
	e			0.8	799	0.1	123	–	–
1.8	γ	1.7	957	0.8	444	0.7	410	0.7	410
	e			1.8	991	1.5	820	–	–
3.0	γ	2.1	697	1.7	574	1.1	369	1.2	410
	e			2.8	922	2.2	717	–	–
5.4	γ	3.6	661	2.3	433	1.7	319	1.7	307
	e			4.2	774	3.6	661	–	–
9.0	γ	4.6	506	3.1	349	2.2	246	2.2	239
	e			5.8	635	4.5	499	–	–
16.2	γ	5.8	361	4.3	266	2.8	175	3.0	186
	e			7.7	474	5.8	361	–	–
27.0	γ	7.4	273	5.5	203	3.7	137	3.9	146
	e			9.4	348	7.7	285	–	–
48.6	γ	9.5	196	7.1	147	4.7	97	5.0	104
	e			12.2	251	10.1	209	–	–
81.0	γ	11.9	146	9.0	112	6.4	79	6.8	84
	e			14.9	184	12.6	156	–	–
145.8	γ	15.7	108	12.3	84	9.0	62	9.3	64
	e			19.4	133	16.3	112	–	–
243.0	γ	20.3	84	16.1	66	12.1	50	12.4	51
	e			25.3	104	20.9	86	–	–
437.4	γ	28.6	65	23.2	53	17.8	41	17.9	41
	e			34.1	78	29.4	67	–	–

The behaviour to γ-irradiation of the corn starch and black pepper is similar, but that of black pepper and paprika, at γ- and electron-beam irradiation is very different.

On that basis it should be concluded that the rheoviscometric measurements could be used for control and identification dry corn-starch, black pepper and paprika, especially for shear rates $\dot{\gamma} \geq 27.0\ s^{-1}$ and irradiation dose, $D > 1$ kGy.

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SAFETY CONSIDERATIONS IN THE OPERATION OF GAMMA PROCESSING FACILITIES*

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The commercial uses of radioactive isotopes cover a very wide range of applications, dominated by the sterilization of medical disposable items, following by plastics' modification, food preservation, waste disinfection, etc. The design features of equipment used to perform all the above duties are basically identical, hence there is no need to discuss their safety features separately. The categories to be considered are:

a. Source Production and Safe Transport Considerations.
b. Worker Safety Considerations.
c. Public Safety and Environmental Considerations.
d. Safe Operation Training.

Cobalt 60 production and encapsulation

Cobalt 59 slugs are 99.9% pure, nickel plated and welded into a zirconium alloy "Inner Capsule". Inner capsules are assembled into Reactor Target Bundles and placed into reactors for activation. After activation, the bundles are transferred into an approved shipping container and transported to the Cobalt Processing Facility, where they are dismantled and further sealed in stainless steel "Outer Capsules".

Capsules are manufactured according to a very strict quality assurance program, which includes verification of incoming materials and chemical analysis of material samples. To improve corrosion resistance, the outer capsules are made from ASTM 316L stainless steel, which, due to its low carbon content, permits welding with minimum carbide precipitation.

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Shipping container design

Shipping containers are designed to meet type B(U) requirements of the International Atomic Energy Agency (IAEA) "Regulation for Safe Transport of Radioactive Materials", Safety Series 6. Type B category is reserved for substantial quantities of radioactive materials, as opposed to Type A which is meant for small, often hand-carried packages, containing small amounts of radioactive materials.

To qualify for certification, each model of a shipping container must undergo destructive testing, as follows:

a. Pierce Test consisting of a free fall from 3 feet high (1 m) onto a 6 inch (15.25 cm) diameter steel pin.
b. Drop Test consisting of a free fall from 30 feet (9.14 m) onto an essentially unyielding surface.
c. Fire Test consisting of exposure to temperatures of 1472 °F (800 °C) for a period of 30 minutes.

On completion of these rigorous destructive tests, the sample container must still maintain a high percentage of its original shielding capacity identified by the applicable standards.

Worker safety considerations

The fundamental criteria for the design of safe radiation processing equipment is to ensure that access to the radiation room is denied while the source is exposed and to ensure that the source cannot be exposed while any personnel are inside the irradiator.

There is over 12 feet (4 m) of water between the top of the source rack and persons working in the irradiation cell when the source is fully lowered. The radiation level inside the cell when the source is lowered, is at the natural background level.

Just outside the shield wall is the operator's station where the source is raised and lowered from a control console. The console also indicated source position, the positions of product carriers inside the cell, as well as various alarms and warning lights.

Public safety and environmental considerations

The public safety issues relative to transport of radioactive isotopes have been addressed in the previous chapters. Following are additional considerations:

a. Radiation from the product. The cobalt 60 gamma photons are well below the threshold of activation energies of all common elements.

b. Escape of radiation from the plant. The process of irradiation takes place inside a cell shielded by approximately 6 feet (2m) thick reinforced concrete walls, constructed as a maze, or labyrinth.
c. Seismic activity. The biological shield and the source storage pool are generally designed as separate structures free to resist lateral ground accelerations independently.
d. Integrity of the C–188 Cobalt 60 pencils. C–188 type cobalt 60 pencils are manufactured and tested under a very stringent quality assurance program. The cobalt slugs are nickel-plated, then seal welded inside zircalloy source elements, and finally seal welded into the stainless steel outer capsule.
e. Fire protection. Temperature sensors are located on the cell roof between the source and the ventilation ducts. These are set to actuate at 160 °F. A smoke detector is mounted on the ventilation filter housing. To prevent the product from igniting and burning, a sprinkler system which circulates pool water and/or a CO_2 system can be provided.
f. Integrity of the source storage pool. The pool is completely lined with stainless steel and is designed with no drains. The water in the pool is continuously circulated through a deionizing unit.

Safe operation training

Planned and well structured training is essential to consistent, safe and efficient irradiator operation. Formal courses and seminars are an effective component of safety training, but they must be complimented by a well structured on-job-training program. This program must be deliberate, scheduled, monitored and documented.

Conclusion

The safety record of the radiation processing industry is excellent. At the present, there are 180 full scale production irradiators in 50 different countries. The total quantity of cobalt 60 in service is approximately 155 million curies. Hundreds of irradiator-years of safe operating experience have been accumulated. To date, there has been no damage to shields that has caused increased radiation exposure to workers or the public.

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ECONOMIC EVALUATION AND UPSCALING OF NUTRITIVE UTILIZATION OF BROAD BEAN USING IRRADIATION TECHNIQUE*

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Abstract

Vicia faba (broad bean) is the most important legume consumed in Egypt as a source of protein. In Egypt the conditions are highly unfavourable for grain storage, insects and microbial spoilage cause great losses.

In the experiment "Egypt's mega gamma 1" Cobalt-60 irradiation was used. The effect of 5 kGy dose in comparison with the unirradiated sample was studied during storage. On the other side the present paper reviews the status of broad beans cultivation in Egypt, annual production, local consumption, export volume and magnitude of annual losses due to infestation by harmful insects.

Economic evaluation of the irradiation processing method for the disinfestation and decreasing the losses in Egyptian broad beans has been dealt with.

Introduction

Vicia faba (broad bean) is considered one of the important crops in Egypt as a source of protein diet.

According to the report of the Ministry of Agriculture (1984–1993) about 40200 feddans (1 feddan = 4000 m^2) are cultivated, which produced about 435 800 tons of broad bean seeds as the average during this period.

In Egypt, the conditions are highly unfavourable for grain storage, insects and microbial spoilage cause great losses. Large number of insects have developed resistance to many insecticides. Hydrogen phosphide (phosphine) is a very common fumigant to treat stored food product, and it is very widely used in developing countries. Recent evidence shows that some insects became resistant to this fumigant, besides safety of the use of insecticides has been questioned. Insecticide residues are

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left in grains and other food products, affecting the consumers. Many of these toxic chemicals are suspected to be carcinogenic. USA has banned ethylene dibromide as fumigant due to its suspected carcinogenecity. No single method can replace the use of toxic fumigants. Disinfestation with radiation shows bright prospect to replace fumigation in controlling insect pests in storage. Another drawback which is associated with fumigation is its improper penetration in all grains, as a result there is a possibility that some insects, particularly those which are developing within the grains are not getting adequate exposure to fumigants.

Irradiation is a fully effective method of killing all insects in their developing stages even within the grains. Being a physical process it leaves no residues. It does not cause any danger to an operator as he is in noway near to the source. The whole process is mechanised. Besides, treatment with radiation is less time consuming compared to chemical treatment and it could be imparted to products in their final packages.

Disinfestation by radiation has proven to be a safe and effective alternative to fumigation. Its implementation to control insects in order to prevent further losses of food in developing countries needs serious consideration (MAHMOUD et al., 1991).

Material and methods

Bean variety

Vicia faba, broad bean (cv. Giza 2) samples were obtained from the Legumes Research Department of the Egyptian Ministry of Agriculture.

Treatments

- The first broad bean samples were irradiated with 5 kGy and stored at room temperature.
- The second broad bean samples were stored without irradiation at room temperature (control).
- Determination of total losses as percentage.

The radiation facility

The ACEL type JS-6500 industrial Cobalt-60 gamma irradiator "Egypt's Mega Gamma 1" has been in operation at National Centre for Radiation Research and Technology (NCRRT) since January 1979 at an initial Cobalt-60 activity of 400 kCi. The plant is furnished with a ventillated concrete biological shield, a water pool for source storage, a principal mechanical conveyor, an extra research channel for pilot

irradiation of high density products, a source pass mechanism with pneumatic pushers and all other devices for interlocks, radiation safety and absorbed dose measurements. The plant design has already been described (ROUSHDY, 1974) and the dosimetric calculation for irradiation processing have been worked out (EL-BEHAY et al., 1981).

Cost consideration for radiation processing

For the present cost evaluation, the following parameters were taken into consideration.

- Plant capital cost including cost of building, concrete biological shield, radioactive source and mechanical installation.
- Plant operating cost including cost of labour, overhead, utilities and radiation dosimeters.
- Plant utilization and source efficiency.
- Rate of radioactive decay and depreciation.
- Internal and rate of return.

Source size

The source size is determined by the maximum throughput rate, the dose required and the efficiency factor for the emitted radiation.

The mathematical relationship is as follows

$$X = 360 \frac{W \cdot n}{D} \text{ kg h}^{-1} \qquad \text{(BRYNJOLFSSON, 1973)}$$

where X = kilograms of product irradiated per hour at a dose of D

D = dose in kilograys

W = kilowatts of radiation from the source (67480 Ci of Co^{60} emit 1 kW of gamma radiation)

n = the efficiency factor, the ratio of the radiation energy absorbed in the product to the radiation energy emitted from the source.

Benefit of irradiation

The benefit of food preservation by irradiation results from the reduction of losses and is given by:

$$B = (X \times P - X(P - P \times R)^{-e}$$

$$B = X \times P \times R \times e$$

where X = is the annual production

e = is the average percentage of the decrease in losses gained through irradiation

P = is the cost per unit weight (mean value) (BOUSSAHA, 1990)

Results and discussion

Beans are part of the diet of Egypt's population. Beans are stored as dry seeds and form an enormous reserve of food, however, this crop is subject to attack by a variety of insects that cause great damage and loss of nutritive foods, which are reflected as one of the causes of malnutrition in Egypt.

The advantages of radiation over fumigation by controlling insect infestation are first, the product can be treated after packaging regardless of the type of packaging used, and secondly, its ability to sterilize insect eggs laid inside kernels of grain, which can escape the action of chemical treatment. There is further advantage that no toxic residue is left in the food when radiation is employed.

The amount produced annually are 460, 451, 466, 382 and 420 thousand metric tons through the years 1989, 1990, 1991, 1992 and 1993, respectively. The results of this evaluation include that net cost of plant operation per ton equals 50 Egyptian pound (LE) (EGYPTIAN MINISTRY OF PLANNING, 1979) for the disinfestation of bean at the dose level of 5 kGy. It is equivalent to a cost of LE 0.05 per kg. KUNSTADT and MARCOTTE (1989) showed that cost analysis, taking into consideration both capital expenditure and annual operating expenditures of gamma processing facilities, indicate costs varying from 0.6 ct/kg for potatoes and onions, 2.5 ct/kg for poultry, to 6 ct/kg for spices, dried seasonings, dried fish, etc. of course numbers are counted on realistic economics of scale. They do not however include the additional savings realized from reduced spoilage, reduced energy costs and improved product quality. Food processor will have to believe that irradiation is cost effective compared to other methods before they become willing to invest in it financially (HALL, 1989).

Estimates of irradiation benefits were based on the average production of bean in the period 1989–1993 and investment costs for Egypt's Mega Gamma 1 facility. The data recorded in Table 1 illustrate the annual income which increased annually by 83 million LE, by an annual ratio of 9.5% from the annual production after deducting the irradiation costs.

Table 1

Economic results of bean irradiation in Egypt

Quantity[a] (t/y)	Irradiation cost (LE/y)	Irradiation cost per unit (LE/t)	Reduction of losses (%)	Unit price of product (LE/t)	Value of % reduction of loss (LE/y)	Benefit of irradiation (LE/y)
435 800	22×10^6	50	12	2000	105×10^6	83×10^6

[a] Average annual production for 1989, 1990, 1991, 1992 and 1993

From these results we conclude that irradiation technology can decrease the annual losses in bean in addition to the other benefits of this technology either from the health side of view of using bean free from microbes, insects, toxines or keeping the nutritional value of the food item as a result of stopping chemical and enzymatic reactions and oxidation processing.

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SUPPRESSION OF OFF-ODOUR IN IRRADIATED CHICKEN USING ADDED DIETARY ANTIOXIDANTS*

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When raw chicken is irradiated, many compounds, both volatile and non-volatile are formed in the tissue. Even at low doses (2.5 kGy), some of these substances give rise to an odour which is unpleasant to many people.

The principal odour components have been identified (PATTERSON & STEVENSON, 1995) and because many are derived from the lipids, increased concentrations of antioxidants, such as α-tocopherol (vitamin E), in the tissue may be effective in conferring resistance to irradiation damage.

Ascorbic acid (vitamin C) is known to complement the antioxidant properties of vitamin E and to lead to enhanced protection against radical attack and lipid peroxidation (WILLSON, 1983). Thus the addition of elevated concentrations of these two antioxidant vitamins has been studied as a possible means of suppressing irradiation odour in raw chicken.

Experimental

Eight groups of chicken were reared on diets containing different combinations of α-tocopheryl acetate and ascorbic acid, plus one group on a control diet containing vitamin E alone at a basic concentration of 50 mg/kg feed.

Equal weights of minced breast and leg tissue from each treatment group were combined separately and four replicates from each were irradiated in sealed flasks at 4 °C.

For each replicate, volatile substances were isolated by a purge and trap procedure and quantitatively analysed by gas chromatography. Peak areas were integrated and summed.

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Table 1

Total peak area and percentage change in irradiation volatiles

Parameters	Treatment group							
	B	Q	N	M	L	K	J	H
	Dietary Vit E/Vit C (mg/kg feed)							
	50/0 (control)	100/50	200/50	200/100	200/200	400/200	400/400	800/800
Mean peak area (sd) ($\times 10^{-3}$/kGy)	34.2 (4.7)	27.2 (4.3)	11.8 (1.4)	12.7 (0.6)	16.6 (1.0)	8.4 (0.3)	11.4 (1.7)	3.8 (0.5)
% Change (treatment vs control, B)		-20.6	-65.5	-62.8	-51.3	-75.5	-66.7	-89.0
Statistical significance		*	***	***	***	***	***	***

Table 2

Statistical significances between treatment means

	B	Q	N	M	L	K	J	H
B	–							
Q	*	–						
N	***	***	–					
M	***	***	NS	–				
L	***	**	**	***	–			
K	***	***	**	***	***	–		
J	***	***	NS	NS	**	**	–	
H	***	***	***	***	***	***	***	–

NS not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Results

Table 1 shows GC integration data for total volatiles (mean and standard deviation) for the replicate samples within each dietary group, together with the percentage change and statistical significance (t-test) of each treatment mean relative to the control. The significance of the differences between the means of all the dietary treatments is shown in Table 2.

Discussion

With one exception, all dietary treatments gave rise to results that were very highly significantly different from those of the control diet (Table 1). In most cases, individual treatments also differed very significantly from one another (Table 2).

When expressed as percentage change, the differences between all the treatment means and the control diet represented reductions in the yields of volatile substances ranging from 20 to almost 90%. The most effective dietary treatment was that containing the greatest additions of the two vitamins, 800 mg of each per kg feed, resulting in the maximum reduction of 89% in volatile production.

Concomitantly with the reduced yield of volatiles, less odour was associated with the samples when assessed by GC-olfactory analysis.

Future work will involve growing chickens on one or more of the enriched diets and using taste panellists to assess the degree of suppression of irradiation off-odour achieved.

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DETECTION OF IRRADIATED FOODSTUFFS*

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Food irradiation has been introduced recently, mainly to reduce spoilage losses and to improve hygienic quality, particularly since 1980, when a Joint FAO/IAEA/WHO Expert Committee (1981) meeting concluded that "the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard". However, the radiation treatment of different foods is now legally accepted in about 40 countries it is still prohibited in others. Consequently, regulatory authorities in all countries appear to be interested in having simple and reliable methods to detect irradiated foods and to check on compliance with labelling regulations.

Several detection methods have been discussed previously but considerable progress has been made recently, particularly due to the actions of the Reference Bureau of the Commission of the European Communities (CEC, Brussels) (RAFFI et al., 1994a) and of the Joint Division of FAO and IAEA (1994) in Vienna. In June 1994, the European Committee for standardization (CEN) created a working group (WGT8) who finally wrote and accepted five protocols (Table 1): two by Electron Spin Resonance (ESR), two by Gas Chromatography (GC) and one by Thermoluminescence (TL). In June 1994 these five protocols have been voted and then transformed into 'pre-norms' by the CEN. We present in this paper two potential applications of ESR (RAFFI & BENZARIA, 1994; RAFFI et al., 1994b) and TL. In case of aromatic herbs (RAFFI et al., 1994b); relative advantages of ESR and TL are discussed, leading to the following conclusions:

- TL done on minerals is always the best method, i.e. the only one leading to a proof; in that case do not remember that the 'official' method requires to re-irradiate the sample and to compare the TL signals before and after this re-irradiation;
- depending on the plant, TL of whole plant or ESR may be the best method for an initial screening.

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Table 1

Draft protocols voted by the CEN

TECHNIQUE	PROTOCOL	No. CEN	SCOPE
E.S.R.	FOOD CONTAINING BONES	No. 48	MEAT and BONES
"	FOOD CONTAINING CELLULOSE	No. 49	STONES, ACHENES, NUTS
TL	FOOD CONTAINING SILICATES	No. 50	HERBS and SPICES
GPC.	FOOD CONTAINING LIPIDS: HYDROCARBONS	No. 46	MEAT
"	FOOD CONTAINING LIPIDS CYCLOBUTANONES	No. 47	MEAT

In case of mussels an oysters (RAFFI et al.) direct TL is not very interesting and requires a second investigation after (re)-irradiation of the sample; but ESR draft protocols were proposed as the relative ESR spectra of the irradiated and non irradiated shells are very different; the minimum dose where identification is possible is lower than 0.1 kGy, i.e. very low with regard to the possible commercial irradiation doses.

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SOME EXAMPLES OF DETECTION OF IRRADIATED FOODSTUFFS*

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In order to facilitate the trade of irradiated foods, regulatory authorities in all countries appear to be interested in having simple and reliable methods to detect foods treated by irradiation and, consequently, to check on compliance with labelling regulations (RAFFI, 1996). Three main methods (Electron Spin Resonance = ESR, Thermoluminescence = TL, Chromatographic Analysis of hydrocarbons induced in lipids = GC) have led to five protocols in course of acceptance by the European Committee of Normalisation (RAFFI, 1996; RAFFI et al., 1994b).

Some examples of application of these methods are given here:

- the shape of the ESR spectra of irradiated and non irradiated foodstuffs are different: spectra of frog legs and raspberries are shown as examples of the two CEN draft protocols (RAFFI et al., 1994b; RAFFI & BENZARIA, 1994) while detection of irradiated meat bones is always easy, detection of irradiated berries is more difficult. The case of dried fruits, such as grapes and papaya, is then shown (RAFFI et al., 1994a) explaining why there is, up to now, no draft CEN protocol.
- The TL intensity differs between the irradiated and the non irradiated samples: the TL measurements carried out on aromatic herbs is first discussed depending on the used protocol (direct TL on the whole product, TL of mineral parts after extraction by the 'official' protocol, without or with re-irradiation, or by direct dry filtration) and on the storage (RAFFI et al., 1994c). The case of shells from oysters and mussels is also discussed.
- In this GC protocol, we have to compare the lipid composition of the foodstuff to the hydrocarbons (induced by irradiation or naturally present): two examples are shown, the case of avocado pears (Fig. 1) where the lipid concentration is relatively low but simple (LESGARDS et al., 1993) and the case of egg powder where the lipid concentration is relatively high but complicate.

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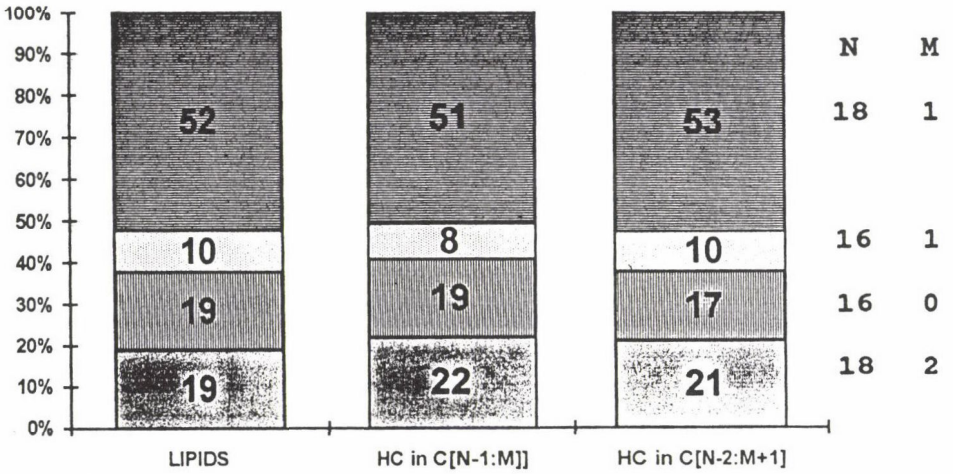

Fig. 1. Histograms showing, in case of irradiated avocado pears, the correlation between the composition of lipids, noted as C[N:M] (N carbone atoms and M double linkages) and those of radioinduced hydrocarbons 'HC' in C[N–1:M] and C[N–2:M+1]

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RADIORESISTANCE OF *LISTERIA MONOCYTOGENES* IN MEAT AND CHEESE*

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Samples of beef, pork and poultry were placed in polyethylene (PE) or polyamide-polyethylene (PA/PE) pouches, inoculated with *L. monocytogenes* and irradiated at 4 °C or −18 °C. Listeria were the least radioresistant in chilled meats packaged in PE and the most resistant in frozen meats packaged in PA/PE (D_{10} values: 225–393 Gy). In the second experiment, samples of 3 ripe cheeses packaged in PA/PE pouches were inoculated with listeria and irradiated at ambient temperature. D_{10} values ranged from 258 to 273 Gy.

Keywords: cheese, *L. monocytogenes*, meat, radioresistance

Listeria monocytogenes is a human and animal pathogen widespread in the environment (KWIATEK et al., 1992). This bacterium is a potentially dangerous organism because being psychrotrophic it can multiply during extended refrigerated storage of the food product (GRAU & VANDERLINDE, 1990). Food borne listeriosis outbreaks have been linked to the consumption of dairy products including raw and pasteurized milk, ice cream and a variety of cheeses, meat and meat products such as pork sausages, hot dogs, cooked ham or hamburger sandwiches as well as chicken and fish (MC LAUCHLIN, 1992).

Various control measures have been applied to reduce the incidence and level of listeria in food including an improvement of GMP by introduction of HACCP concept (FARBER, 1991; TOMPKIN, 1990), however a complete elimination of the pathogen by those means seems to be impossible. Irradiation has been successful in removing certain non-spore-forming pathogens from meat and poultry and was also recommended by WHO (1988) for elimination of *L. monocytogenes* from several foods.

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The aims of the study were: (a) to determine the effect of irradiation, temperature and packaging on radiation resistance of *L. monocytogenes* in minced beef, pork and poultry and (b) to determine the radiation resistance of *L. monocytogenes* in three different ripe cheeses (the most popular in Poland), sliced and vacuum packaged in polyamide-polyethylene (PA/PE) pouches.

1. Materials and methods

In the first experiment samples of beef (lean top round), pork (sirloin) and poultry (breast muscles) were ground and decontaminated by gamma irradiation at a dose of 15 kGy at 0 °C. Directly before experiments the sterile meat was aseptically placed (9±0.05 g) in Stomacher 400® polyethylene bags (PE) or polyamide--polyethylene (PA/PE) bags widely used in the Polish food industry for meat, meat products, fish and cheese (code "Multiseven" 78 TOP, WIPAK, Finland).

All samples were inoculated with *L. monocytogenes*. The mixture of five *L. monocytogenes* strains isolated from beef, pork and poultry were used in the study. All isolates were obtained from the Polish State Veterinary Institute. After inoculation the samples were sealed in the presence of air (PE bags) or under vacuum (PA/PE bags) and stored overnight at +4 or -18 °C before irradiation. The samples were irradiated, in triplicate, with cobalt-60 gamma rays, with 0, 0.5, 1.5, 2.0 and 2.5 kGy in chilled (+4 °C) or frozen (-18 °C) state, at a dose rate of 100 Gy min^{-1}. Fricke dosimetry was used for calibration (IAEA, 1977).

Directly after irradiation the number of surviving listeria in each sample was determined by the ten-fold dilution method followed by plating onto Columbia Agar Base (Oxoid). Plates were incubated at 37 °C for 48 h. Statistical analyses of bacterial counts transformed to the base 10 were conducted. The results were elaborated by using analysis of variance. D_{10} values (doses required for reduction of bacterial population by 1 log unit) were calculated using regression analysis (MARTIN, 1972).

In the second experiment, samples of sliced ripe cheeses (Edamski, Gouda, Podlaski) were inoculated with *L. monocytogenes* (a mixture of three strains isolated from milk), vacuum packaged in PA/PE bags, and irradiated with increasing doses of gamma rays at ambient temperature. After irradiation the number of surviving listeria in each sample was determined by the ten-fold dilution method and plating onto Oxford Agar (Oxoid). Aerobic Plate Counts (APC) were determined using a solid medium prepared according to Polish Standard (PN, 1993). All plates were incubated at 30 °C for 72 h. The results were elaborated in a similar way as in the first experiment.

2. Results and discussion

The overall analysis of variance indicated that in the first experiment, radiation dose, temperature of irradiation and the packaging material significantly influenced ($P<0.01$) the listeria counts. The effect of kind of meat was not large enough to be statistically significant at $P<0.01$.

D_{10} values obtained in this study for all kinds of meat (Table 1) indicate that the bacteria were much more sensitive to irradiation in chilled meats than in frozen meats. Radiation resistance of *L. monocytogenes* was lower in samples packaged in oxygen permeable film than in meats irradiated in PA/PE bag which is a better barrier against oxygen. This was also found by other authors (MULDER, 1976; THAYER & BOYD, 1991). The effect of temperature of irradiated samples on survival of listeria was stronger than the effect of packaging material under the conditions of this experiment. The lowest D_{10} value (224.66 Gy) was found for pork packaged in polyethylene bag and irradiated at 4 °C, the highest one (393.43 Gy) for beef packaged in a polyamide--polyethylene bag and irradiated at -18 °C (Table 1).

Table 1

Effect of irradiation temperature and packaging on radiation resistance of L. monocytogenes *in meat*

Temperature of the sample (°C)	Packaging	D_{10} (Gy)	Correlation coefficient
	Beef		
+4	PE	264.94	-0.980
+4	PA/PE	322.42	-0.996
-18	PE	375.68	-0.977
-18	PA/PE	393.43	-0.983
	Pork		
+4	PE	224.66	-0.990
+4	PA/PE	255.32	-0.966
-18	PE	337.70	-0.975
-18	PA/PE	340.27	-0.984
	Poultry		
+4	PE	235.89	-0.989
+4	PA/PE	280.68	-0.985
-18	PE	331.35	-0.977
-18	PA/PE	331.78	-0.982

PE: polyethylene
PA/PE: polyamide/polyethylene

The D_{10} values for *L. monocytogenes* obtained in this study are lower than those reported by HUHTANEN and co-workers (1989) who found that the mean D_{10} value at 2–4 °C for the destruction of *L. monocytogenes* in mechanically deboned chicken meat was 0.77 kGy ranging from 0.59 to 1.03 kGy. Also PATTERSON (1989) reported that D_{10} values for several *L. monocytogenes* strains irradiated in poultry mince at ambient temperature were 0.417–0.553 kGy depending on strain and the plating medium.

The obtained data indicate that *L. monocytogenes* is much more sensitive to irradiation than *Salmonella* spp. in similar studies (SZCZAWIŃSKA, 1994).

As shown in Table 2, the radiation resistance of listeria in all examined cheeses was very similar. D_{10} values were slightly lower than those obtained for meats vacuum packaged in PA/PE, that could be related to the higher temperature during irradiation. The results for APC (Table 2) indicate that the inherent microflora of ripe cheeses is much more resistant to radiation than *L. monocytogenes*. Similar observations were also found by other authors, however D_{10} values for *L. monocytogenes* in Camembert cheese reported by BOUGLE and STAHL (1993) were higher and amounted to 0.5 kGy.

3. Conclusions

1. Temperature of irradiation and packaging materials exert significant effect on radiation resistance of bacteria and these factors should be taken into account when calculating the doses of radiation necessary for elimination of bacterial pathogens from food.

2. Doses of radiation applied to eliminated salmonellae from food would also be sufficient to remove *L. monocytogenes*.

Table 2

Radiation resistance of L. monocytogenes *and inherent microflora in cheese*

Type of cheese	*L. monocytogenes*		Aerobic plate count	
	D_{10} (Gy)	Correlation coefficient	D_{10} (Gy)	Correlation coefficient
Edamski	257.74	–0.984	322.71	–0.979
Gouda	261.02	–0.984	413.92	–0.958
Podlaski	273.46	–0.979	370.79	–0.984

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Acta Alimentaria, Vol. 25 (4), pp. 403–405 (1996)

BOOK REVIEWS

Essentials of the Microbiology of Foods.
A Textbook for Advanced Studies

D. A. A. MOSSEL, J. E. CORRY, B. STRUIJK, R. M. BAIRD et al.

John Wiley & Sons, Chichester, 1995, xxxv + 699 pages

The first author, equally famous as scientist and educator, who is perhaps the internationally best known food microbiologist of our age, will make together with his renowned co-authors a long-lasting impetus to microbiological studies on food. The wealth of factual information condensed in the book and its ecological approach on assessment and assurance of microbiological safety and quality of foods makes this publication an outstanding document of this important area of food science. To all readers – particularly post-graduate students deeply interested in food microbiology and related subjects – the book gives not only a professional library in itself but also a view of looking at microbiological problems which helps understanding connections and motivates vocation. This is proved to the reviewer who is using the book happily at his University with a class of Ph.D. students in food science.

The book provides tremendous amount information which are still presented in a readable form condensed by the authors after the critical review of a vast literature. The proportions of this effort are shown by the fact that the list of references makes 202 double-coloumned pages, taking almost one third of the impressively large volume. Considering the lead time necessary to publication of a book, the list of references contains also a lot of recent items.

This compresensive treatise covers the whole field of modern food microbiology. The scientific breadth of the book can be illustrated already by the list of only the main chapters, each of them divided into many sub-headings:

Part I.:

1. General Principles of Assuring the Microbiological Safety, Quality and Acceptability of Foods.
2. Major Taxonomic and Determinative Characteristics of Organisms of Importance in Foods.
3. Factors Affecting the Fate and Activities of Microorganisms in Foods.
4. Diseases of Microbial Origin Transmitted by Foods.
5. The Mechanism and Principles of Control of Microbial Spoilage of Foods.

Part II.:

6. The Control of Microbiological Safety and Quality of Foods.
7. The Microbiological Monitoring of Foods.
8. Evaluation of the Efficacy of Measures to Ensure Wholesomeness and Quality of Food by Assessing Compliance with Reference Values ("Standards").

Part III.:

9. Recommended Routine Procedures for the Microbiological Examination of Foods.

A sufficiently detailed subject index adds to the utility of this well structured material. However, considering the frequency of use and handling a book as useful as this, the present bulky single volume is not a very convenient form because its regular use causes soon a physical tear and wear by its own weight. Dividing it into e.g. three more slim volumes could be advantageous provided the triplicate binding and the necessary repetitive listing of a part of the references would not increase the total price unbearably.

Summing up, this outstanding book should be on the shelves of every laboratory working in the field of applied and food microbiology and be available to graduate students, lecturers and researchers involved in food sciences and technology. With its wealth of data in the form of tables, figures and reference values within the text, is serves as a very useful reference to quality assurance personnel both in the food industry and food control institutions.

J. FARKAS

Fermented meats

G. CAMPBELL-PLATT & P. E. COOK (Eds)

Blackie Academic & Professional; Chapman & Hall 1995, 242 pages

Preservation by fermentation is one of the oldest food technologies and yet it continues to play an important role in meat preservation, involving starter cultures with controlled conditions.

The preservation of meats by fermentation depends on the interaction of various microecological factors. This subject is an important but relatively specialised area of microbiology and food technology. It is becoming increasingly important to gain a better understanding of meat fermentations and how these processes can be controlled.

Fermented meats are popular foods in many parts of the world and diverse range of products is available, therefore it was necessary to review the present state of knowledge in the field of meat fermentation.

This book begins (Chapter 1) with a general discussion on the properties of meat then gives a world perspective of fermented meats in Chapter 2.

This leads into more specialised chapters on the history (Chapter 3) and diversity of fermented meats, followed by chapters on the microbiology, chemistry and preservation aspects of this technology.

Bacterial and fungal fermentation is discussed in Chapters 4 and 5. Starter cultures the tools of controlled fermentation are introduced in Chapter 6.

An overview of the stable ad safe sausage fermentation is given in Chapter 7. Chapter 8 is devoted to the chemistry of fermented sausages. Chapter 9 presents basic information about fungal toxins in meats.

The final chapter (Chapter 10) considers fermented meat production and consumption in the European Union. We hope that fermented meat production of those countries not yet members of the EU but world-famous of their salamis might be included in the next revised edition as well.

Each of the ten chapters provides important, valuable information and updated references on this historical nevertheless newly invented topic. In spite of the differences between sausages fermented world-wide this book provides information for all those interested in meat fermentation.

This volume is an excellent and up-to-date overview of the subject for food scientists and microbiologists in the academic sector.

D. BÁNÁTI

Principles of modified atmosphere and sous vide product packaging

J. M. FARBER & K. L. DODDS (Eds)

Technomic Publishing AG, Basel, 1995, 468 pages

This new book of publisher presents in one volume the modified atmosphere packaging and sous vide food preservation and packaging techniques and technologies. The book is dealing with the basic and significant knowledges on food quality assurance by modified atmosphere and sous vide preservation processes. Both these technologies extend the shelf life of foods, while usually avoiding the use of various preservatives.

The book contains 15 chapters written by well known experts of food preservation and packaging.

The first chapter (Introduction) introduces the fundamentals and history of modified atmosphere packaging and sous vide technology. The perspective and current status of MAP and sous vide technology are in the second and third chapter with special regard to North America and Europe. The microbiological concerns associated with MAP and sous vide products are in chapter four. The fresh and cooked meat and poultry products packed in MAP are the objectives of fifth and sixth chapters, while the fish and shellfish products in sous vide and modified atmosphere packs are in the seventh one. We can read about the principles and practice of MAP of horticultural commodities in chapter eight, and in case of bakery and pasta products in chapter nine. The tenth chapter gives a review about the past, present and future of sous vide technique. The eleventh chapter is dealing with the potential use of additional hurdles to increase the microbiological safety of MAP and sous vide products. The main aspects of gas absorbents and generators for present and future of MAP are in chapter twelve. There is a well detailed interpretation of the application of HACCP for MAP and sous vide products in the next chapter. Potential use of time-temperature indicators as an indicator of temperature abuse of MAP product is described by the authors of chapter fourteen. The title of the last chapter is the regulation and guidelines regarding the manufacture and sale of MAP and sous vide products.

At the end of the book a very up-to-date list of references is given to help the researchers to deep in details. The Index of subjects can help to use the book which is recommended for food scientists, microbiologists and technologists, but university and high school students and teaching staff as well as to all who are interested in packaging.

I. VARSÁNYI

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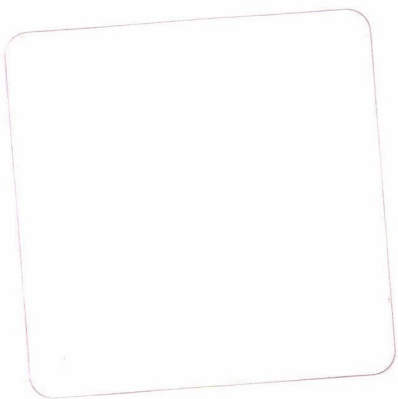

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